Study of the Transfer RNAs Coded by T2, T4, and T6 Bacteriophages*

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T2, T4, and T6 bacteriophage tRNAs coding for arginine, leucine, proline, isoleucine, and glycine were isolated under conditions of short term and long term infection of Escherichia coli B cells. The corresponding phage tRNA species were examined for sequence homology by RNA-DNA hybridization analysis and by their relative behavior on reversed phase chromatography. The results indicate that all three T-even phages code for similar tRNA molecules. Weiss et al. (1) had originally shown that %-labeled T4 tRNAs hybridized equally well to T2 and T4 DNAs. Waters and Novelli (3) and Tillack and Smith (4) independently suggested the presence of phage-specific tRNAs in T2-infected cells. Kim and Davidson (5) carried out a study on the sequence relationship of T2, T4, and T6 DNAs by using the heteroduplex method. They concluded that all of the T-even phages contained tRNA genes, some of which were homologous.

In the present study, we infected Escherichia coli B cells separately with the three different T-even phages and then attempted to examine the RNA extracts for phage-specific tRNA species by comparing their sequence homology by RNA-DNA hybridization analysis and to study their relative behavior on reversed phase chromatography. The results presented here indicate that all three T-even phages code for several different tRNA species; some are homologous, others are not, and not all of the same tRNA species are coded by each bacteriophage. Reversed phase chromatography showed the presence of isoacceptor tRNAs for each phage aminoacyl-tRNA species. Pulse-chase experiments for \[32P\]tRNA\*G\* suggest that the multiple isoacceptor species observed derive from the intracellular modification of a single tRNA\*G\* gene product.

Based on criteria of nucleotide composition, DNA sequence homology, serological cross-reactivity, morphology, and genetic recombination and complementation, T2, T4, and T6 bacteriophages are considered to be closely related. The discovery of tRNAs coded by the T4 genome (1, 2) made it likely that all three T-even phages code for similar tRNA molecules. Weiss et al. (1) had originally shown that \[35S\]-labeled T4 tRNAs hybridized equally well to T2 and T4 DNAs. Waters and Novelli (3) and Tillack and Smith (4) independently suggested the presence of phage-specific tRNAs in T2-infected cells. Kim and Davidson (5) carried out a study on the sequence relationship of T2, T4, and T6 DNAs by using the heteroduplex method. They concluded that all of the T-even phages contained tRNA genes, some of which were homologous.

In the present study, we infected Escherichia coli B cells separately with the three different T-even phages and then attempted to examine the RNA extracts for phage-specific tRNA species by comparing their sequence homology by RNA-DNA hybridization analysis and to study their relative behavior on reversed phase chromatography. The results presented here indicate that all three T-even phages code for several different tRNA species; some are homologous, others are not, and not all of the same tRNA species are coded by each bacteriophage. Reversed phase chromatography showed the presence of isoacceptor tRNAs for each phage aminoacyl-tRNA species. Pulse-chase experiments for \[32P\]tRNA\*G\* suggest that the multiple isoacceptor species observed derive from the intracellular modification of a single tRNA\*G\* gene product. A preliminary report on a part of this work has been presented (6).

MATERIALS AND METHODS

Growth and Purification of Phages - Phages T2 and T4 were grown in TYN medium (1 liter contained 10 g of Difco tryptone, 2 g of yeast extract, 6 g of NaCl) with E. coli B as host. T6 was grown in TYN medium, except that it contained 5 g of yeast extract and 10 g of NaCl, per liter, and E. coli B strain RH288 (obtained from Dr. A. Markowitsch, The University of Chicago) was used as host. Cells were infected at a density of \(7 \times 10^8\) per ml, with a multiplicity of infection of 0.2 for T2 and T4, and of 2 for T6. Prior to infection, tryptophan was added to a concentration of 100 \(\mu\)g/ml. Infection was allowed to proceed for 3 h at 37\(^\circ\), and lysis was aided by the addition of CHCl\(_3\). Phages were purified from crude lysates by differential centrifugation, and then by banding in CsCl. After dialysis against pH 7.6 buffer (10 mM potassium phosphate, 1 mM MgSO\(_4\), and 150 mM NaCl), phage preparations were stored at 4\(^\circ\) in the presence of small amounts of CHCl\(_3\).

Isolation of Phage DNA - The purified phage preparations were diluted with phage buffer to contain approximately 30 A\text{260} units/ml and extracted three times with an equal volume of redistilled phenol equilibrated with 1 x SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 6.0). The final aqueous phase was dialyzed extensively against 1 x SSC, pH 6.0, and stored at 4\(^\circ\).

Isolation of RNA from Phage-infected Cells - The hosts and conditions for T2, T4, and T6 infection were as mentioned above, except that the multiplicity of infection was approximately 5 plaque-forming units per cell. The infection periods were 13 min for T2 and T6 and 16 min for T4, both at 37\(^\circ\), unless otherwise specified. Infection was terminated by the addition of 0.01 volume of 1 M NaN\(_3\), rapid chilling, and centrifugation. Nucleic acid was isolated from the infected cell pellets by phenol extraction and then deacylated as described previously (7). The nucleic acid preparation was stored to stand in 1 M NaCl at 4\(^\circ\) overnight; the precipitate which appeared was removed by centrifugation. The 1 M NaCl soluble fraction was diluted 4-fold, passed over Whatman DEAE-32, and nucleic acid was eluted with 0.8 M NaCl, 0.05 M NH\(_4\) acetate (pH 5.5). Appropriate fractions were combined and treated with 2 volumes of ethanol, the precipitate collected was dissolved in 0.01 M NaCl and used as crude infected tRNA. Crude tRNA was also prepared from phage-infected cells to which chloramphenicol (50 \(\mu\)g/ml) was added 6 h min after infection. The infection was terminated after 1 h of incubation; rifampicin (200 \(\mu\)g/ml) was added 5 min prior to termination.

Preparation of Phage-specific tRNAs - The crude infected tRNA

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preparation (described above) for each T-even phage was used for the isolation of phage specific tRNAs, free of host tRNA, by annealing to the appropriate homologous phage DNA and recovery of the hybridized RNA, as described elsewhere (8).

**Aminoacyl Synthetase and tRNA Charging - Aminoacyl synthetase** was prepared from soluble cell extracts of E. coli MRE-600 by a procedure used previously (9). Aminoacylations were done in reaction mixtures containing the following (per millilitre): 100 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 2 mM ATP, 5 mM KCl, 2.5 mM 2-mercaptoethanol, 500 μg of crude tRNA, 70 to 250 μCi of a single 3H-amino acid or 15 to 25 μCi of a single 14C-amino acid, and 950 μg of E. coli B tRNA and lowering of the pH to approximately 5. The reaction mixture was incubated at 37°C for 5 min, followed by ethanol precipitation. The precipitate was collected by centrifugation, dissolved in 1 ml of 0.01 M NaCl, and dialyzed extensively against the same solution.

The specific activity of radioactive 3H- and 14C-amino acids (New England Nuclear) ranged from 23.6 to 83.4 Ci per mmol and 0.10 to 0.30 Ci per mmol, respectively.

**Aminoacyl-tRNA Hybridization with DNA - Hybridizations of tRNA and DNA were carried out on membrane filters (type B-6, Schleicher and Schuell, Inc.) which were impregnated with approximately 40 μg of DNA purified from one of the three T-even phages. Radioactive 3H-aminoacyl-tRNAs were annealed with the DNA filters at 37°C for 4 h in 2 × SSC containing 50% formamide (pH 5.5). The exact annealing conditions and determination of the radioactive "fixed" to membrane filters, including a wash procedure with T1 RNase treatment, were as reported before (1).

**Reversed Phase Chromatography - Phage-specific tRNA charged with a single 3H-L-aminocain acid was subjected to chromatography in the RPC-5 system of Pearson et al. (10). Columns (0.6 × 25 cm) containing a phosphate adsorbent were equilibrated at room temperature with a buffered solution (pH 4.7) containing 0.4 mM NaCl, 0.03 mM sodium acetate, 0.01 M magnesium acetate, and 0.002 M 2-mercaptoethanol. A mixture of phage 3H-aminoacyl-tRNAs (20,000 to 110,000 cpm total) and E. coli B 14C-aminoacyl-tRNA (charged with the same amino acid and containing a similar amount of radioactivity) was loaded on the column in equilibrium solution. The column was washed with 20 ml of the same solution, and then eluted with a linear NaCl gradient (pH 4.7). The total amount of RNA loaded on the column did not exceed 1 mg.

The gradient volume was 210 ml, and 1-ml fractions were collected during each run. Samples were taken from the gradient fractions for direct measurement of radioactivity by liquid scintillation counting.

**Isolation of T4 32P-tRNA from T4am61-infected Cells - E. coli B cells were grown in 45 ml of low phosphate medium (approximately 0.1 mm phosphate), as described by Pinkerton et al. (11). At a density of 3 × 10⁸ per ml, cells were infected with T4am61 phage (MOI-6); 6 min after infection, one-half of the infection medium was removed, NaCl was added, and the cells were rapidly chilled and pelleted by centrifugation. To the remaining half, cold phosphate buffer (final concentration, 0.1 M) and rifampicin (200 μg/ml) were added. Incubation was continued for 30 min, after which infection was terminated, and the cells were collected by centrifugation.
T-even Bacteriophage tRNAs

Fig. 2. Hybridization of T-even phage [3H]glycyl-tRNA to T-even phage DNAs. A, hybridization with crude T2 [3H]glycyl-tRNA. The specific activity of [3H]glycine was 43 Ci/mm, and the charged tRNA preparation contained 55 × 10⁶ cpm/ml. The conditions for annealing were the same as those described for Fig. 1. B, hybridization with crude T4 [3H]glycyl-tRNA. The specific activity and conditions for annealing were the same as in A, except that the charged tRNA preparation contained 29 × 10⁶ cpm/ml. C, hybridization with crude T6 [3H]glycyl-tRNA. The specific activity and conditions for annealing were the same as in A, except that the charged tRNA preparation contained 50 × 10⁶ cpm/ml.

Fig. 3. Hybridization of T-even phage [3H]arginyl-tRNA to T-even phage DNAs. A, hybridization with crude T2 [3H]arginyl-tRNA. The specific activity of [3H]arginine was 27.5 Ci/mm, and the charged tRNA preparation contained 20 × 10⁶ cpm/ml. The conditions for annealing were the same as for Fig. 1, except that the annealing volume was 2.0 ml. B, hybridization with crude T4 [3H]arginyl-tRNA. The specific activity and conditions for annealing were the same as in A, except that the charged tRNA preparation contained 18 × 10⁶ cpm/ml. C, hybridization with crude T6 [3H]arginyl-tRNA. The specific activity and conditions for annealing were the same as in A, except that the charged tRNA preparation contained 25.6 × 10⁶ cpm/ml.

Fig. 4. Hybridization of T-even phage [3H]isoleucyl-tRNA to T-even phage DNAs. A, hybridization with crude T4 [3H]isoleucyl-tRNA. The specific activity of [3H]isoleucine was 30 Ci/mm, and the charged tRNA preparation contained 88 × 10⁶ cpm/ml. The conditions for annealing were the same as for Fig. 1, except that the annealing volume was 2.0 ml. B, hybridization with crude T6 [3H]isoleucyl-tRNA. The specific activity and conditions for annealing were the same as in A, except that the charged tRNA preparation contained 12 × 10⁶ cpm/ml, and the annealing volume was 0.9 ml.

Fig. 5. Hybridization of T-even phage [3H]prolyl-tRNA to T-even phage DNAs. A, hybridization with crude T2 [3H]prolyl-tRNA. The specific activity of [3H]proline was 16 Ci/mm, and the charged tRNA preparation contained 8.8 × 10⁶ cpm/ml. The conditions for annealing were the same as for Fig. 1. B, hybridization with crude T4 [3H]prolyl-tRNA. The specific activity and conditions for annealing were the same as in A, except that the charged tRNA preparation contained 13.2 × 10⁶ cpm/ml.

infected cells, charged with [3H]proline, showed no hybridization to T6 DNA, suggesting the absence of this tRNA gene in the T6 chromosome.

Table I summarizes the results presented in Figs. 1 to 5 and shows the presence (+) or absence (−) of tRNA sequence homology for the five amino acid tRNA species coded by each T-even phage. The absence of a detectable phage tRNA species is indicated by the letters ND.

Chromatography of Short Term Infected Phage tRNAs—The crude tRNA recovered from 13- to 16-min phage-infected cells was used for the preparation of phage-specific tRNAs as described under "Materials and Methods." The phage-specific tRNAs were charged with a single 3H-aminoacid and subjected to chromatography in the RPC-5 system of Pearson et al. (10). Fig. 6 shows the chromatography profile for T2, T4, and T6 tRNAs charged with [3H]leucine. The profile of Escherichia...
charged with $\textit{[3H]}$glycine showed very similar profiles (Fig. 7). In the case, the major species eluting in a position almost identical to the $\textit{T2}$ and $\textit{T6}$ tRNAs, different from the $\textit{TRNA}^{\textit{A20s}}$ isoacceptors of $\textit{E. coli}$. When $\textit{T4}$ tRNAs were charged with $\textit{[3H]}$leucine, they repeatedly gave poorly defined chromatographic profiles.

Chromatography of the three T-even phage-specific tRNAs charged with $\textit{[3H]}$glycine showed very similar profiles (Fig. 7). Two isoacceptor glycyl-tRNA species were seen in each case, the major species eluting in a position almost identical to the major glycyl-tRNA species of $\textit{E. coli}$. Contamination of the phage tRNA preparations with $\textit{E. coli}$ tRNAs is unlikely, since the chromatographic profiles of phage tRNAs charged with other amino acids (Figs. 6, 8, and 10) did not show any significant coincidence with the charged $\textit{E. coli}$ tRNA markers.

Multiple tRNA$^{\textit{A20s}}$ isoacceptors appear for all three T-even phage tRNAs when these preparations are subjected to RPC-5 chromatography (Fig. 8). Although the profiles for $\textit{T4}$ and $\textit{T6}$ tRNAs were nearly identical, the profile for $\textit{T3}$ arginyl-tRNA was distinctly different. In the latter case, higher salt concentrations were required for the elution of $\textit{T2}$ tRNA$^{\textit{A20s}}$ isoacceptors.

Figs. 9 and 10 show the chromatography profiles for $\textit{T2}$ and $\textit{T4}$ $\textit{[3H]}$prolyl-tRNA$^{\textit{a}}$, and for $\textit{T4}$ and $\textit{T6}$ $\textit{[3H]}$isoleucyl-tRNA$^{\textit{a}}$, respectively. No profiles are given for $\textit{T2}$ tRNA$^{\textit{A20s}}$ or $\textit{T2}$ tRNA$^{\textit{A20s}}$ since these phage tRNA species were not detected. The elution profiles for $\textit{T4}$ and $\textit{T6}$ tRNAs were qualitatively similar, showing two tRNA$^{\textit{A20s}}$ isoacceptor species; the relative amounts of the T2 and T4 isoacceptors were different, however. For isoleucyl-tRNA, $\textit{T4}$ tRNA showed two distinct RPC-5 peaks, but $\textit{T6}$ tRNA gave only one.

Chromatography of Long Term Infected Phage tRNAs—Phage-specific tRNAs were also prepared from cells infected with T-even phages for 60 min in the presence of chloramphenicol. For most of the "long term" phage tRNA preparations, RPC 5 profiles similar to those for the "short term" phage tRNA species were observed. However, as shown in Fig. 11, the chromatographic profile of tRNA$^{\textit{G20s}}$, isolated from 60-min infected cells, was significantly different from that for short term infected tRNA$^{\textit{G20s}}$ (Fig. 7) in that an extra isoacceptor peak appeared which eluted at lower salt concentrations; in addition, there were quantitative changes in the same isoacceptors observed previously. It is possible that the altered 60-min infected tRNA$^{\textit{G20s}}$ profile might be attributable to some nonspecific effect of chloramphenicol (14), but similarly altered tRNA$^{\textit{G20s}}$ profiles were found for phage tRNAs prepared from 60-min $\textit{T4am61}$-infected cells, defective in $\textit{T4}$ lysozyme production, in the absence of chloramphenicol. The appearance of multiple isoacceptor tRNA species might result from the enzymatic modification of a single tRNA gene transcript; this possibility was explored for $\textit{T4}$ tRNA$^{\textit{G20s}}$ by pulse-chase experiments with inorganic $\textit{32P}$.

$\textit{E. coli}$ cells infected with $\textit{T4am61}$ were exposed to $\textit{32P}$, between 6 and 12 min after infection (in the absence of chloramphenicol); a portion of these cells was removed and subjected to phenol extraction. To the remaining cells, an excess of cold inorganic phosphate and rifampicin was added, and they were allowed to incubate for a total infection time of 60 min. The "chased" and "nonchased" infected cells were proc-

### Table I

| Phage tRNA species | T2 | T4 | T6 |
|-------------------|----|----|----|
| $\textit{T2 tRNA}^{\textit{A20s}}$ | + | - | - |
| $\textit{T4 tRNA}^{\textit{A20s}}$ | - | + | + |
| $\textit{T6 tRNA}^{\textit{A20s}}$ | - | + | + |

* ND = absence of a detectable phage tRNA species.

![Fig. 6. Reversed phase chromatography of short term infected T even phage leucyl-tRNAs.](http://www.jbc.org/Downloaded from http://www.jbc.org/)

**Figure 6.** Reversed phase chromatography of short term infected T even phage leucyl-tRNAs. Phage-specific $\textit{T2}$, $\textit{T4}$, and $\textit{T6}$ tRNAs derived from 3- to 16-min infected cells and free from $\textit{E. coli}$ tRNA (see "Materials and Methods"), were used for charging with $\textit{[3H]}$leucine, and commercial $\textit{E. coli}$ B tRNA (Schwarz BioResearch) was used for charging with $\textit{[14C]}$leucine. Radioactive leucyl-tRNA preparations were subjected to RPC-5 chromatography as described under "Materials and Methods" and eluted with a linear NaCl gradient, 0.4 M to 0.9 M, pH 4.7. The fractions collected (1 ml) were analyzed directly in a Nuclear-Chicago Mark I scintillation spectrometer set for double counting, and the counts per min obtained were corrected for spillover. Recovery of applied radioactivity ranged from 50 to 90%. A, a mixture of $\textit{T2}$ $\textit{[14C]}$leucyl-tRNA and $\textit{E. coli}$ B $\textit{[14C]}$leucyl-tRNA. B, $\textit{T4}$ $\textit{[3H]}$leucyl-tRNA. C, $\textit{T6}$ $\textit{[3H]}$leucyl-tRNA. $\textit{E. coli}$ B $\textit{[3H]}$leucyl-tRNA was also included in B and C as marker (not shown); its profile was essentially the same as shown in A.
cessed in the same way for the isolation of [32P]tRNAc1r (see under "Materials and Methods"). The [32P]tRNAc1r profile (Fig. 12A) for the 12-min infected cells (nonchased) was almost identical with that found for 12-min infected T4 [3H]glycyl-tRNA (Fig. 7). Fig. 12B shows that, after an extended chase period, the major radioactive tRNAc1r peak of Fig. 12A is reduced, whereas the minor peak of Fig. 12A is considerably enhanced. At the same time, an additional radioactive peak appears which elutes earlier than the other two isoacceptors, at slightly lower salt concentrations. In the absence of rifampicin (Fig. 12C), cells infected with the mutant phage T4am61 and continuously exposed to [32P] for 60 min produce multiple tRNAC1r isoacceptor species on RPC-5, coincident with the nonchased and chased isoacceptors shown in Fig. 12, A and B.

**DISCUSSION**

These studies demonstrate that all three T-even phages carry genes coding for tRNAs, and that this information is expressed following phage infection of *Escherichia coli*. Of the five amino acid tRNA species examined (leucine, glycine, arginine, proline, and isoleucine), T4 RNA extracts contained all five species, whereas T2 and T6 extracts were missing tRNAC1r and tRNATPs, respectively. For similar amino acid tRNA species expressed by each of the three T-even phages, a significant degree of sequence homology was detected, except in the case of T2 tRNAAm which showed no homology with either T4 or T6 tRNAAm. This difference between the tRNATp species of the T-even phages was also reflected by RPC-5 chromatography, in which the profile of T2 arginyl-tRNA was distinctly different from those obtained with T4 and T6 arginyl-tRNAs; the latter two profiles resembled each other rather closely.

The quantitative differences observed for the hybridization of the various charged tRNA species to the different phage DNAs is not altogether clear. In some cases, saturation of T-even DNA with homologous tRNA species was not achieved; hence, interpretation of these results is difficult. For those reactions in which near saturation or saturation was achieved, hybridization to T2 DNA appeared to be significantly higher than to T4 and T6 DNA (e.g., leucyl- and prolyl-tRNAs). These results might indicate the presence of multiple tRNA gene copies in the T2 genome; however, the hybridization results with other tRNA species (e.g., glycyl-tRNAs) showed relatively small differences among the three phage DNAs. It is possible, therefore, that the variations in the levels of hybridization observed for each DNA were due to some intrinsic factor in the annealing procedure itself.

For the three T-even phage RNA extracts, multiple tRNA isoacceptors were detected on RPC-5 for the five different amino acid tRNA species. In some instances, coincident profiles for phage and *E. coli* tRNAs were observed on RPC-5 chromatography. One would expect that, if the phage-specific tRNAs were contaminated with host tRNAs, all of the phage [3H]aminoacyl-tRNA species examined on RPC-5 would also have shown [3H]radioactivity coincident with that of *E. coli* [14C]aminoacyl-tRNAs used as markers; this was not the case.

![Fig. 7](http://www.jbc.org/)

**Fig. 7.** Reversed phase chromatography of short term infected T-even phage glycyl-tRNAs. The phage-specific and *Escherichia coli* (E.C.) B tRNAs described for Fig. 6 were used for charging with [3H]- and [14C]-labeled glycine, respectively. The procedure for chromatographic analysis was the same as for Fig. 6, except that a 0.4 M to 0.65 M NaCl linear gradient was employed for elution. A, a mixture of T2 [3H]glycyl-tRNA and E. coli B [14C]glycyl-tRNA. B, T4 [3H]glycyl-tRNA. C, T6 [3H]glycyl-tRNA. Both B and C also contained E. coli B [14C]glycyl-tRNA as marker (not shown). Recovery of the applied radioactivity was greater than 90% in each run.

![Fig. 8](http://www.jbc.org/)

**Fig. 8.** Reversed phase chromatography of short term infected T-even phage arginyl-tRNAs. The phage specific and *Escherichia coli* (E.C.) B tRNAs (described for Fig. 6) were used for charging with [3H]- and [14C]-labeled arginine, respectively. The procedure for chromatographic analysis was the same as for Fig. 6, except that a 0.4 M to 0.8 M NaCl linear gradient was employed for elution. A, a mixture of T2 [3H]arginyl-tRNA and E. coli B [14C]arginyl-tRNA. B, T4 [3H]arginyl-tRNA. C, T6 [3H]arginyl-tRNA. Both B and C contained E. coli B [14C]arginyl-tRNA as marker (not shown). Recovery of the applied radioactivity was greater than 85%.
I. I I I I1 11 11 II
T2 [3H] PROLYL-tRNA @
40 50 60 70 SO PO 100
I10 120 130 140 150 160
FRAGMENT NUMBER
FIG. 9. Reversed phase chromatography of short term infected T-even phage prolyl-tRNAs. The phage-specific and Escherichia coli (E.C.) B tRNAs described for Fig. 6 were used for charging with "H- and 14C-labeled proline, respectively. The procedure for chromatographic analysis was the same as for Fig. 6, except that a 0.5 M to 0.8 M NaCl linear gradient was employed for elution. A, a mixture of T2 [3H]prolyl-tRNA. B also contained E. coli B [14C]prolyl-tRNA as marker (not shown). Recovery of the applied radioactivity was greater than 85%.

The coincidence of certain phage and E. coli tRNA isoacceptor species on RPC-5 is fortuitous and does not indicate sequence homology, since E. coli tRNAs do not compete with phage tRNAs for hybridization sites on phage DNA (15).

The appearance of isoacceptor phage tRNA species, in short and long term infected tRNA preparations, raises the question whether these isoacceptors represent separate gene products or derive from the enzymatic modification of a single tRNA gene copy. Some insight into this question, at least for tRNA^Leu^, was obtained by the 32P pulse-chase experiment illustrated in Fig. 12. This experiment indicates that the major RPC-5 tRNA^Leu^ species present 12 min after infection is modified and converted into several other isoacceptor peaks during prolonged incubation of the T4am61-infected cells. This modification cannot be attributed to chloramphenicol (14) since none was present. In the absence of rifampicin and with continuous labeling (Fig. 12C), T4am61 infection results in the appearance of multiple tRNA^Glu^ isoacceptor species similar to those seen after infection with T4 wild type in the presence of chloramphenicol. It is possible that the erratic chromatographic profiles observed for phage tRNA^Leu^ (Fig. 6) are partially related to this tRNA modification process, which might also explain the latent appearance of multiple T2 tRNA^Leu^ isoacceptors initially reported by Waters and Novelli (3). It is not known whether the phage tRNA modifications represent

Fig. 10. Reversed phase chromatography of short term infected T-even phage isoleucyl-tRNAs. The phage-specific and Escherichia coli (E.C.) B tRNAs described for Fig. 6 were used for charging with "H- and 14C-labeled isoleucine. The procedure for chromatographic analysis was the same as for Fig. 6, except that a 0.4 M to 0.75 M NaCl linear gradient was employed for elution. A, a mixture of T4 [3H]isoleucyl-tRNA and E. coli B [14C]isoleucyl-tRNA. B, T6 [3H]isoleucyl-tRNA. B also contained E. coli B [14C]isoleucyl-tRNA as marker (not shown). Recovery of the applied radioactivity was greater than 85%.

Fig. 11. Reversed phase chromatography of long term infected T4 phage glycyl-tRNA. T4 phage-specific tRNA, derived from 60-min infected cells in the presence of chloramphenicol and rifampicin, and free from Escherichia coli (E.C.) tRNA (see "Materials and Methods"), was used for charging with "Hglycine. The conditions for chromatography were the same as for Fig. 7, and E. coli B [14C]glycyl-tRNA was included as marker.
T-even Bacteriophage tRNAs

Fig. 12. Reversed phase chromatography of T4 [32P]tRNA's labeled under pulse-chase conditions. [32P]-labeled tRNA was isolated from cells infected with T4am61, which had been exposed to [32P] under various conditions. The labeled tRNA species were isolated as described under "Materials and Methods" and subjected to chromatography as outlined in Fig. 7. Escherichia coli (E.C.) B [3H]glycyl-tRNA served as a marker for each of the above runs. A, [32P]tRNA isolated from T4am61-infected cells pulsed with [32P], from 6 to 12 min of the infection period. B, [32P]tRNA isolated from T4am61-infected cells, pulsed with [32P], as in A, and then chased in the presence of a large excess of cold inorganic phosphate and rifampicin for a total infection time of 60 min. C, [32P]tRNA isolated from cells infected with T4am61 phage and exposed to [32P] from 6 to 60 min of infection, in the absence of rifampicin. E. coli B tRNA charged with [3H]glycine was included in each chromatography but is shown only in A.

a continuation of the normal alterations that occur during tRNA maturation, or whether they are simply a consequence of tRNA degradation and turnover. In either event, some of the modified phage tRNAs are still capable of being enzymatically esterified with amino acids. The pulse-chase experiment suggests that the multiple isoacceptor peaks seen on RPC-5 chromatography for phage tRNAs, and possibly for the other phage tRNA species as well, originated from a single gene tRNA transcript. Nevertheless, as was shown for T5 phage tRNAs (9, 16, 17), the presence or absence of multiple tRNA isoacceptor gene loci in T-even phage DNAs has not been firmly resolved.

The similarity in the phage tRNA species expressed by the three T-even phages supports the information obtained previously which indicates the close genetic relationship between these three bacteriophages. Nevertheless, some differences in phage tRNA synthesis and sequence homology have been found; this emphasizes that the degree of genetic relatedness for different biological systems is often a function of the particular biochemical parameter examined. Except for highly selected bacterial strains (18), bacteriophage tRNAs have not been shown to be required for phage growth. Our findings are consistent with the view that variations in genetic information between highly related organisms are most likely to be found in those genes which are not essential for growth and survival.

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