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Isoaccepting Species of Serine tRNA Coded by Bacteriophage T5st0

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By aminoacyl-tRNA-DNA hybridization and chromatographic analysis, evidence was provided that the bacteriophage T5st0 codes for two $tRNA^{Ser}$ species. Trinucleotide- or polynucleotide-stimulated binding experiments assigned the codons UCC or UCU to these two $tRNA^{Ser}$ species. They also suggested that the synthesis of these two $tRNA^{Ser}$ species does not modify the reading capacity for codons less used in *Escherichia coli* F and corresponds to a different situation compared with the T4-coded tRNA's.

By hybridization experiments using phage DNA and infected *Escherichia coli* tRNA aminoacylated in vitro, Scherberg and Weiss (18) have demonstrated the existence of 14 different tRNA species coded by T5 phage genome and 5 by T4 phage genome. Furthermore, McClain et al. (9) have identified, by electrophoresis of [^{32}P]RNA from T4-infected bacteria on polyacrylamide gel, eight T4-coded tRNA's which have each been found to be a single species.

No isoaccepting tRNA's coded by phage have been detected until recently, when Chen et al. (4) showed, by RNA-DNA hybridization and chromatographic analysis, that T5 induces the synthesis of $tRNA_m^{Met}$ and $tRNA_f^{Met}$.

In the present paper we report that hybridization is achieved with a high efficiency between one strand of T5st0 DNA and aminoacylated tRNA from infected *E. coli* F, providing evidence that more than one molecule of seryl-tRNA hybridized with one molecule of T5st0 DNA. Furthermore, chromatography on reversed-phase column permits us to separate two serine tRNA species which are shown to hybridize with distinct T5st0 DNA sites.

Scherberg and Weiss (19) reported that some T4 phage tRNA species tend to prefer code words that are less recognized by their host. The situation seems to be different in the case of T5st0 phage $tRNA^{Ser}$'s, as is shown by their codon recognition study.

MATERIALS AND METHODS

Growth of bacteria. Erlenmeyer flasks (2 liters) containing 200 ml of medium consisting of 0.1 M Tris-hydrochloride (pH 7.4), 2 mM potassium phosphate buffer (pH 7.4), 50 mM NaCl, 1 mM $MgSO_4$, 0.003 mM $FeSO_4$, 20 mM $(NH_4)_2SO_4$, 1.1 mM $CaCl_2$, 0.2% Casamino Acids (Difco), and 0.4% glucose were inoculated with fresh cultures of *E. coli* F to about 0.5 $\times$ 10^8 cells/ml and incubated at 37 C.

To prepare T5st0-infected bacteria, *E. coli* F were grown to 4 $\times$ 10^8 cells/ml and then infected at a multiplicity of 5 for 20 min. Uninfected and infected bacteria were harvested by centrifugation, frozen, and stored at -20 C. Growth and purification of T5st0 bacteriophage were performed as described by Labedan et al. (7).

tRNA preparation. Cells were suspended in 10 mM acetate buffer (pH 5) containing 10 mM $MgCl_2$, 0.5% bentonite, and 0.5% sodium dodecyl sulfate and stirred with an equal volume of phenol at 50 C for 4 min. The aqueous and phenol layers were separated by centrifugation, and the latter was reextracted with acetate buffer containing 10 mM $MgCl_2$. Aqueous layers were combined, and a further phenol treatment was performed for 15 min at room temperature. RNA was precipitated from the aqueous layer by the addition of potassium acetate (pH 5) to a final concentration of 2% and of 2 volumes of ethanol. After 2 h at -20 C, the precipitate was collected by centrifugation and dissolved in TM buffer (10 mM Tris-hydrochloride, pH 7.2-10 mM $MgCl_2$). Precipitation of high-molecular-weight RNA and stripping of the tRNA preparation were accomplished by the procedure of Von Ehrenstein and Lipman (22). The preparations were further purified by chromatography on Sephadex G-100. Fractions containing tRNA were pooled and concentrated by pervaporation; tRNA was precipitated and dissolved in TM buffer.

Aminoacyl-tRNA preparation. The mixture of aminoacyl-tRNA synthetases was prepared from uninfected or infected bacteria by the method of Muench and Berg (13). tRNA was charged with L-[^{14}C]- or L-[^{3}H]amino acids in an incubation mixture containing 1 mg of tRNA per ml, 0.025 mM radioactive L-amino acids, 0.01 mM other L-amino acids, 0.1 M Tris-hydrochloride, pH 7.5, 15 mM $MgCl_2$, 5 mM ATP, and about 1 mg of aminoacyl-tRNA synthetase preparation per ml. After 20 min of incubation, potassium acetate (pH 5) was added to a final concentration of 2% and aminoacyl-tRNA's were

isolated by phenol extraction, precipitated by ethanol, and dissolved in 1 mM potassium acetate (pH 5). The extent of aminoacylation was determined by the acid-precipitable radioactivity.

Chromatography of seryl-tRNA on RPC5. The RPC5 system was used as described by Pearson et al. (15). Chromatography of aminoacylated tRNA was performed at room temperature. Columns were equilibrated with a solution containing 10 mM potassium acetate, pH 4.5, 0.4 M NaCl, 10 mM $MgCl_2$, and 1 mM 2-mercaptoethanol. The columns were then loaded with the aminoacylated tRNA samples (less than 2 mg). Aminoacyl-tRNA was eluted with a 900-ml linear salt gradient (0.45 M to 1.0 M NaCl) containing the other constituents of the equilibration solution. About 500 fractions (1.8 ml per fraction) were collected. Aliquots from the fractions were analyzed for radioactivity. Fractions containing the different seryl-tRNA species were pooled, put into a dialysis bag, and concentrated against solid polyethylene glycol. The concentrated solution was dialyzed against 1 mM potassium acetate (pH 5) and used for hybridization experiments or binding tests.

Isolation of T5st0 H and L strands. DNA was extracted, denatured, and sedimented as previously described by Bujard and Hendrickson (3) except that only 800 μg of DNA was layered onto each tube and the sucrose gradient contained 2% Sarkosyl NL 97. Gradients were collected and the optical density at 254 nm was continuously recorded by an ISCO-UA4 absorbance monitor. Fractions containing H strand or the fragments of L strand were pooled, dialyzed against 0.1 mM Tris-hydrochloride, pH 8-1 mM NaCl-0.1 mM EDTA, concentrated by pervaporation, and dialyzed extensively against the same solution as above. The concentration of DNA solutions was determined assuming that, in these conditions of ionic strength and pH, a 1-μg/ml solution has an absorbance at 260 nm of 0.0274.

tRNA-DNA hybridization. In a first procedure RNA-DNA hybridization was carried out on nitrocellulose membranes (Millipore HAWP, pore size 0.45 nm) impregnated with denatured *E. coli* or T5st0 DNA by the procedure of Gillespie and Spiegelman (5). *E. coli* DNA was isolated according to Marmur (11) and T5st0 DNA was extracted by a phenol technique analogous to the method of Mandel and Hershey (10). [^{3}H]aminoacyl-tRNA's were annealed in the presence of the disk in 2× SSC (0.15 M NaCl plus 0.015 M sodium citrate) and 50% formamide solution (pH 5.3) for 3 h at 33 C (21). After hybridization each side of the filter was washed with 50 ml of 2× SSC. In a second procedure, hybridization was carried out in solution containing T5st0 H or L strand isolated as described above. Annealing conditions were the same as in the first procedure except for the pH (see below). The incubated mixture was diluted 100 times with 6× SSC and filtered on nitrocellulose membranes previously washed with 6× SSC. It was necessary to lower the formamide concentration to 0.5% to retain DNA on filters. After filtration, the filters were washed with 50 ml of 6× SSC solution. In both procedures [^{14}C]DNA was added to check the retention of DNA on the filters. Treatment with ribonuclease was omitted, as it had no influence on our experimental results. Finally the radioactivity fixed on the filter was determined.

Binding of seryl-tRNA to ribosomes in the presence of polyribonucleotides or trinucleotides. *E. coli* ribosome preparations and binding experiments were performed as described by Roy and Söll (16). Trinucleotide UCG was synthetized from UpC (8, 20). The incubation mixture (0.05 ml) contained 0.1 M Tris-acetate, pH 7.2, 0.05 M KCl, 0.02 M magnesium acetate, 2.5 absorbance units of ribosomes at 260 nm polyribonucleotides or trinucleotides, and seryl-tRNA as indicated for each experiment. Incubation was carried out at 25 C for 20 min.

Materials. Radioactive L-amino acids were purchased from the CEA, Saclay, France. Specific activities (Ci/mmol) were as follows: [^{3}H]amino acids: Arg, 19, Ile, 26; Met, 7; Ser, 43; Tyr, 43; Val, 30; [^{14}C]Ser, 0.099. The counting efficiency for ^{3}H was 20%; when ^{14}C and ^{3}H isotopes were used together, their counting efficiencies were, respectively, 55 and 18%. Plaskon impregnated with Adogen 464, used for the RPC5 system, was purchased from Miles Laboratories. UpC was purchased from Sigma, and random copolymers poly(U,C) and poly(A,G,U) were obtained from Miles Laboratories.

RESULTS

Hybridization of T5st0 aminoacyl-tRNA's with T5st0 H and L strands. T5 bacteriophage has a nonpermuted linear duplex DNA; one strand is intact (H strand), whereas the other (L strand) contains several interruptions at genetically determined positions (1, 3, 6, 21). Sedimentation of denatured DNA in a sucrose gradient allows one to separate the H strand from the different L strand fragments. By hybridization studies using aminoacyl-tRNA's obtained from infected bacteria and H strand or the fragments of L strand, M. J. Chen, J. Locker, and S. B. Weiss (Fed. Proc. **33**:1280, 1974) have claimed that most of the T5 tRNA's, if not all, are transcribed from the H strand. Before this assertion was reported, we were attempting to localize the T5 tRNA genes applying the same technique to the T5st0 phage, a thermostable deletion mutant of wild-type T5 (17). Experiment data presented are in agreement with the statement of Chen et al. (Fed. Proc. **33**:1280, 1974) and furthermore focused our attention on seryl-tRNA from T5-infected bacteria.

Fractions obtained from sedimentation of denatured DNA in neutral sucrose gradient and containing H strand and the fragments of L strand were pooled as indicated (Fig. 1), concentrated, and dialyzed against a low-ionic-strength buffer. tRNA extracted from infected bacteria was aminoacylated in vitro by six different [^{3}H]amino acids. The different extents of aminoacylation (Table 1) may be explained

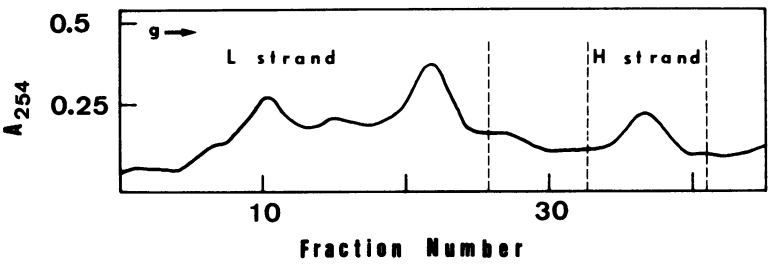

FIG. 1. *Sedimentation of T5st0 DNA in neutral sucrose gradient. Fractions containing the H strand and the fragments of the L strand were pooled as indicated. Sedimentation was performed as described in Materials and Methods. A_{254}, Absorbance at 254 nm.*

TABLE 1. *Annealing of individually charged [^{3}H]aminoacyl-tRNA's from uninfected and infected bacteria with H and L strand of T5st0 DNA*[a]

Source of tRNA	[^{3}H]-amino acids	Extent of amino-acylation (pmol/mg of tRNA)	Deacyl-ation after anneal-ing	Charged tRNA added (pmol)	[^{3}H]amino acids fixed on filter corrected from the blank (pmol × 10^3)		[^{3}H]aminoacyl-tRNA annealed (pmol/ pmol of DNA)		Ratio (A/B)
					H strand	L strand	H strand (A)	L strand (B)	
Infected bac-teria	Ser	920	47	30.6	124	12	0.490	0.017	28.8
	Ile	340	29	11.3	29	15	0.087	0.018	4.6
	Met	640	47	21.3	89	49	0.258	0.055	4.7
	Tyr	730	59	24.3	16	15	0.061	0.022	2.8
	Val	1,730	24	57.6	21	17	0.079	0.024	3.2
	Arg	1,560	59	52.0	28	25	0.105	0.035	3.0
Uninfected bacteria	Ser			30.5	10	19	0.034	0.026	1.3
	Ile			12.8	4.9	14	0.019	0.020	0.95

[a] tRNA was charged with different L-[^{3}H]amino acids. Each annealing reaction contained one [^{3}H]aminoacyl-tRNA, H strand, L strand, or no DNA in a final volume of 5 ml at pH 7.0. DNA retained on the filter was in the range of 0.25 to 0.35 pmol for the H strand and 0.68 to 0.88 for the L strand. Blanks without DNA were generally in the range of 2×10^{-3} to 7×10^{-3} pmol. Other details are described in Materials and Methods.

either by inadequate charging conditions for each tRNA species or by variable relative amounts of each tRNA species. Table 1 shows the results of annealing experiments performed in solution. This method is, according to preliminary tests, 20 to 50% more efficient than with DNA fixed on nitrocellulose filters. In addition, as H and L strands are usually 80 to 90% pure with respect to complementary sequences (Henckes et al., submitted for publication), this method allows the contaminating DNA to hybridize with the complementary strand, thus decreasing its annealing with aminoacyl-tRNA's. The results given in Table 1 show that hybridization level is more important with H strand than with L strand (the ratio is 28.8 in the case of the seryl-tRNA, 2.8 to 4.7 for the others). Hybridization with the L strand seems to be nonspecific since the amount of seryl- or isoleucyl-tRNA from infected bacteria hybridized with this strand is of the same order of magnitude as the amount of seryl- or isoleucyl-tRNA from uninfected bacteria hybridizable with either the L or H strand (Table 1). Therefore we can conclude that the genes for the tested tRNA's are localized on the H strand.

In these annealing experiments performed at pH 7 the hybridization level for individual aminoacyl-tRNA was lower than one molecule per H strand. This may be due to the important deacylation of aminoacyl-tRNA which occurred during the incubation period, as determined by the acid-precipitable radioactivity of control samples. Results were not corrected by taking into account the extent of deacylation, since the rate of deacylation during annealing reaction may be different for T5-coded tRNA's and *E. coli*-coded tRNA's. On the other hand, the

aminoacyl-tRNA concentration may not be high enough to saturate the tRNA gene sites. Finally, charging conditions for each amino acid may not be optimal. For these reasons further experiments were performed in the case of seryl-tRNA, which gave the highest level of hybridization.

Hybridization of T5st0 seryl-tRNA with T5st0 H strand. We tested the hybridization efficiency at a lower pH (5.3) and as a function of the seryl-tRNA concentration (Fig. 2). In this experiment hybridization values were not corrected for a deacylation of about 10%. From the values obtained after deduction of blanks without DNA, it is obvious that more than one molecule of seryl-tRNA can be annealed per molecule of H strand: at the highest seryl-tRNA input value, 0.13 pmol of T5st0 H strand hybridized 0.2 pmol of seryl-tRNA. To determine the saturation value, data were expressed by the Scatchard plot representation (12) (Fig. 3). This may represent the hybridization of two tRNA^{Ser} species which are in different concentrations in the mixture of seryl-tRNA's and bind to two DNA sites. The extrapolated curve gives a maximum extent of hybridization of about two molecules of seryl-tRNA per molecule of T5st0 H strand.

Results were not corrected for the tRNA bound in an unspecific way to the DNA. But hybridization experiments using *E. coli* seryl-tRNA and H or L strand, just as hybridization experiments using T5-infected *E. coli* seryl-tRNA and L strand, allow us to consider that unspecific hybridization is about 7% of the hybridization value. Furthermore, the addition of 10-fold or more excess of tRNA from uninfected bacteria did not lower the T5-infected *E. coli* seryl-tRNA hybridization value by more than 15% (data not shown). Accordingly, even by neglecting corrections for deacylation and by taking into account unspecific hybridization of

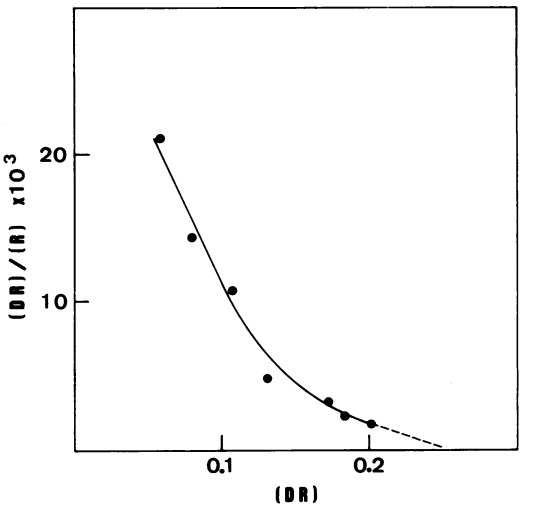

FIG. 3. *Data from Fig. 2 (hybridization corrected from blanks without DNA) analyzed by the Scatchard method. R, Input seryl-tRNA (pmol/0.4 ml); DR, fixed seryl-tRNA (pmol).*

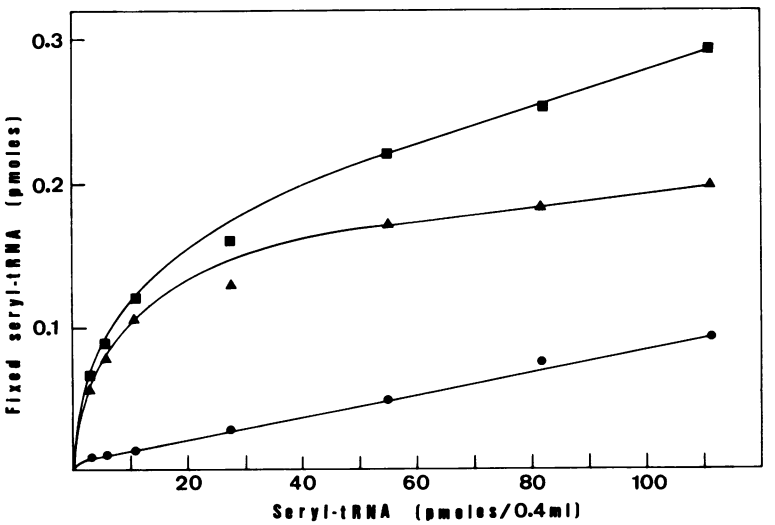

FIG. 2. *Hybridization of [^{3}H]seryl-tRNA from infected bacteria with T5st0 H strand as a function of the seryl-tRNA concentration. Annealing was performed at pH 5.3 in a final volume of 0.4 ml containing 0.130 pmol of T5st0 H strand (■) or no DNA (●) and various amounts of seryl-tRNA. Hybridization corrected from blanks without DNA is indicated (▲). Other details are described in Materials and Methods.*

7 to 15% we can conclude that more than one molecule of seryl-tRNA can hybridize with one molecule of H strand DNA.

Reversed-phase chromatography of seryl-tRNA obtained from uninfected and infected bacteria. tRNA's isolated from T5-infected and exponential-phase uninfected *E. coli* F were aminoacylated in vitro with radioactive L-serine and co-chromatographed on an RPC5 column (1 by 45 cm). No significant difference was found in the extent of aminoacylation nor in the RPC5 elution pattern of tRNA aminoacylated with synthetases from either uninfected or infected bacteria. The elution pattern (Fig. 4) shows that seryl-tRNA obtained from infected bacteria presents two additional discrete peaks eluted in front of the salt gradient and one additional shoulder. The two additional peaks account, in the elution order, for about 7 and 4.5% of the total T5-infected *E. coli* seryl-tRNA. However, elutions patterns varied according to preparations: the first peak represented 7 to 22% of the total seryl-tRNA, and the second represented 3 to 9% in our preparations.

Identification of seryl-tRNA obtained from infected bacteria and separated by RPC5 columns. (i) Hybridization with T5st0 and E. coli DNA. Seryl-tRNA isolated from infected bacteria was chromatographed on an RPC5 column. A better resolution was obtained with a higher column (1 by 55 cm) (Fig. 5). Seryl-tRNA was separated in different fractions as indicated. After concentration as described above, seryl-tRNA was hybridized either with T5st0 DNA or with *E. coli* DNA fixed on nitrocellulose membranes (Table 2). The procedure of isolation and concentration of the different seryl-tRNA fractions leads to about 50% deacylation. Therefore the hybridization values are largely underestimated. Results show that seryl-tRNA from fractions 1 and 2 only hybridizes with T5st0 DNA, seryl-tRNA from fraction 7 hybridizes only with *E. coli* DNA, and seryl-tRNA from pooled fractions (3 + 4 and 5 + 6) hybridizes with both T5st0 and *E. coli* DNA. These results indicate that $tRNA^{Ser}$ corresponding to peaks 1 and 2 and to a part of all the other fractions except fraction 7 are coded by T5st0 bacteriophage.

(ii) Saturation curves with T5st0 DNA. Figure 6 shows the results of hybridizing increasing amounts of seryl-tRNA from the differents RPC5 column fractions with a constant amount of T5st0 DNA. Hybridization experiments were performed in the presence of *E. coli* tRNA to prevent unspecific annealing with T5st0 DNA. Except for fraction 6 containing very little T5-coded $tRNA^{Ser}$, curves ap-

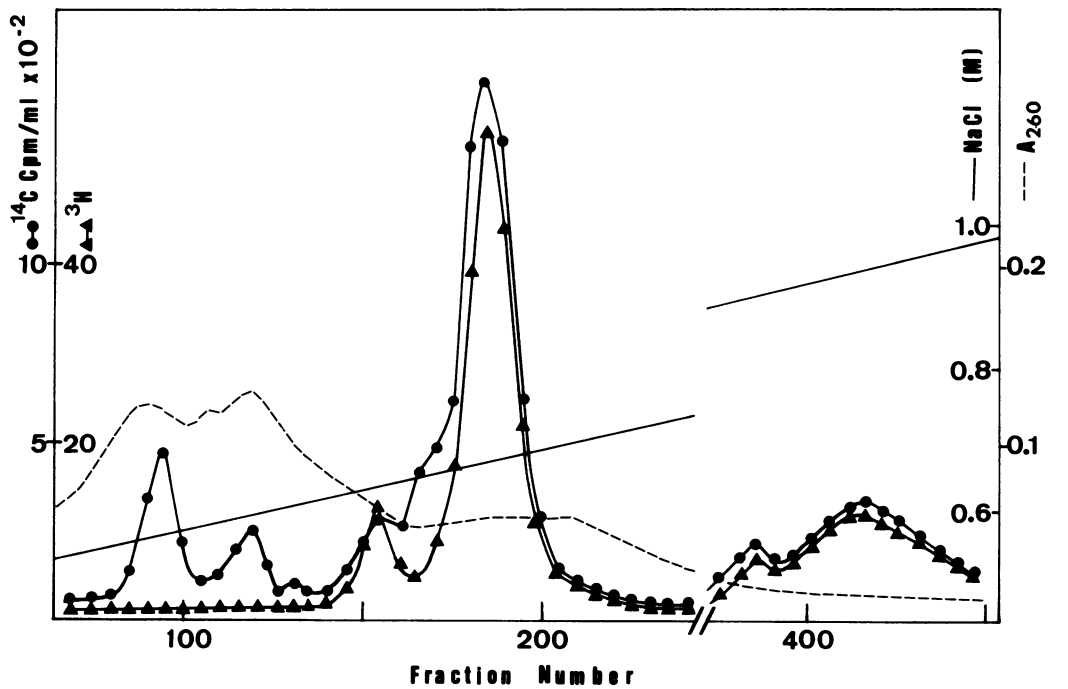

FIG. 4. *RPC5 co-chromatography of [^{3}H]seryl-tRNA from uninfected bacteria (▲) and [^{14}C]seryl-tRNA from infected bacteria (●). Details are described in Materials and Methods. A_{260}, Absorbance at 260 nm.*

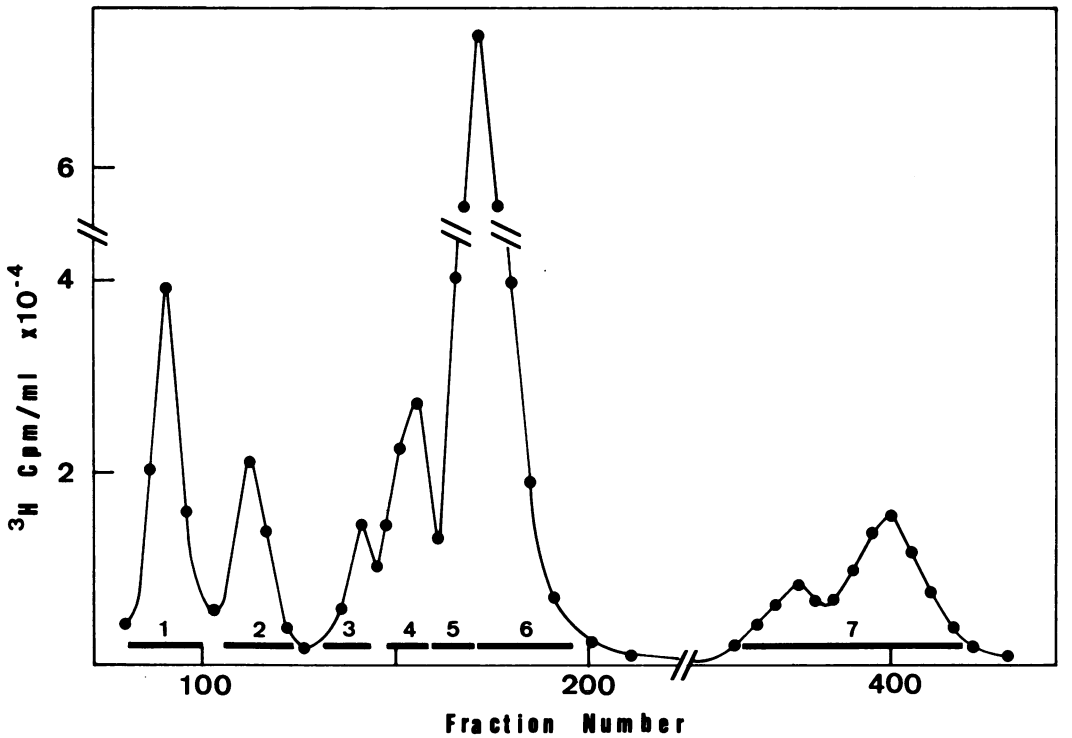

FIG. 5. *RPC5 chromatography of [^{3}H]seryl-tRNA from infected bacteria. Fractions were pooled as indicated. Details are described in Materials and Methods.*

TABLE 2. *Hybridization of [^{3}H]seryl-tRNA from infected bacteria fractionated on an RPC5 column with T5st0 DNA and E. coli DNA*[a]

RPC5 fractions	Seryl-tRNA input (pmol)	Seryl-tRNA fixed on filter (pmol × 10^3)			Seryl-tRNA fixed on filter corrected from the blank (pmol × 10^3)		Ratio (A/B)
		T5st0 DNA	*E. coli* DNA	No DNA	T5st0 DNA (A)	*E. coli* DNA (B)	
1	22	390	24	12	378	12	31.5
2	18.7	632	19	10	622	9	69.1
3 + 4 (pooled)	12	85	22	11	74	11	6.7
5 + 6 (pooled)	22	123	32	10	113	22	5.1
7	23	18	60	10	8	50	0.16

[a] Annealing was performed at pH 5.3 in a final volume of 2 ml containing one nitrocellulose membrane with 100 μg of fixed T5st0 or *E. coli* DNA or no DNA and seryl-tRNA separated by RPC5 column as shown in Fig. 5. Other details are described in Materials and Methods.

proached nearly the same plateau value. Therefore T5-coded tRNA^{Ser} is more abundant in the first half of the major peak than in the second half. The difference observed in the plateau levels of the different fractions could be due to other uncontrolled factors as, for example, the rate of deacylation.

(iii) Competition experiments. To determine whether the different fractions of T5-coded tRNA were hybridized with distinct DNA sites, competitive hybridization was performed between these different fractions for T5st0 DNA. Table 3 shows that saturating concentrations of seryl-tRNA from fractions 1 and 2 or 2 and 4 hybridized with T5st0 DNA in an additive fashion; however, seryl-tRNA from fractions 1 and 4, 3 and 4, 4 and 5, and 1 and 5 hybridized in a competitive manner. Another experiment (Fig. 7) showed that no significant competition occurs between seryl-tRNA from fraction 2 and

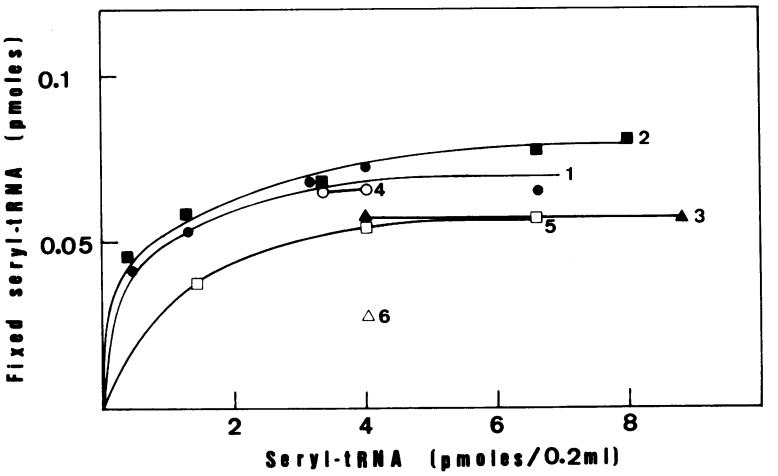

FIG. 6. *Hybridization of [^{3}H]seryl-tRNA from infected bacteria separated on RPC5 column with T5st0 DNA as a function of the seryl-tRNA concentration. Fractions were numbered as indicated in Fig. 5. Annealing was performed at pH 5.3 in a final volume of 0.2 ml containing one nitrocellulose membrane with 17 μg of fixed DNA, 70 μg of tRNA from uninfected bacteria, and various amounts of seryl-tRNA. Other details are described in Materials and Methods.*

TABLE 3. *Competitive hybridization assay between [^{3}H]seryl-tRNA from infected bacteria fractionated on an RPC5 column with T5st0 DNA*[a]

RPC5 fractions	Seryl-tRNA input (pmol)	Seryl-tRNA fixed on filter (pmol × 10^3) (A)	Fixed seryl-tRNA calculated on the assumption of additive hybridization (pmol × 10^3) (B)	Ratio (A/B)
1	6.3	75		
2	4.2	96		
3	4.4	57		
4	4.2	65		
5	6.6	55		
1 + 2	6.3 4.2	159	171	0.93
1 + 4	6.3 4.2	87	140	0.62
2 + 4	4.2 4.2	151	161	0.94
3 + 4	4.4 4.2	77	122	0.63
4 + 5	4.2 6.6	78	120	0.65
1 + 5	6.3 6.6	82	130	0.68

[a] Fractions of [^{3}H]seryl-tRNA were separated on an RPC5 column as shown in Fig. 5 and hybridized at saturating concentration with 17 μg of T5st0 DNA fixed on nitrocellulose membrane (diameter, 12 mm). Annealing was performed at pH 5.3 in a final volume of 0.2 ml.

deacylated tRNA from fraction 1 or from pooled fractions 3, 4, and 5. However, hybridization of seryl-tRNA from fraction 1 was inhibited by deacylated tRNA from pooled fractions 3, 4, and 5. These results suggest that the different chromatographic behavior of T5-coded tRNA^{Ser} from fraction 1 and from fractions 3, 4, and 5 depends either on post-transcriptional modifications or on conformational changes which could occur during the isolation procedure. However, T5-coded tRNA^{Ser} species from fractions 1, 3, and 4 and 5 on one hand and from fraction 2 on the other possess rather distinctive nucleotide sequences and therefore are transcribed from different sites on T5st0 DNA.

Coding properties of the different seryl-tRNA species. The coding response of the seryl-tRNA obtained from infected bacteria and separated on an RPC5 column was determined by their polynucleotide- or trinucleotide-stimulated binding to ribosomes (14). tRNA^{Ser} codons are UCA or UCG, UCC or UCU, and AGC or AGU (16). Table 4 shows the coding response of the seryl-tRNA contained in the different peaks obtained after RPC5 chromatography. These results show that UCC or UCU are the T5-specific tRNA^{Ser} codons. *E. coli* tRNA^{Ser} species, however, are not resolved on the RPC5 column. From the binding values and from the proportion of each peak in the RPC5 elution pattern, we can estimate that about 70% of the total seryl-tRNA from infected bacteria recognize UCC or UCU codons.

We also tested the whole mixture of seryl-tRNA's from uninfected and infected bacteria to compare the two seryl-tRNA populations. The binding of the seryl-tRNA was proportional to the seryl-tRNA concentration with the condi-

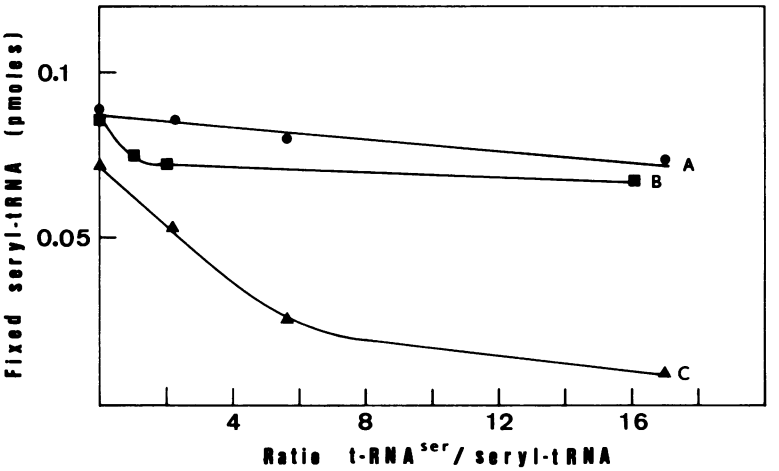

FIG. 7. *Competitive hybridization assay between [^{3}H]seryl-tRNA and deacylated tRNA from infected bacteria separated on an RPC5 column with T5st0 DNA. (A) Seryl-tRNA from fraction 2 and deacylated tRNA from pooled fractions 3, 4, and 5. (B) Seryl-tRNA from fraction 2 and deacylated tRNA from fraction 1. (C) Seryl-tRNA from fraction 1 and deacylated tRNA from pooled fractions 3, 4, and 5. tRNA^{Ser} concentration was determined by the serine acceptance activity.*

TABLE 4. *Template specificity in binding [^{3}H]seryl-tRNA from infected bacteria separated on RPC5 column to ribosomes*[a]

RPC5 fractions	Seryl-tRNA input (pmol)	Seryl-tRNA bound to ribosomes (pmol)			
		Minus template	Plus template		
			Poly (U,C)	Poly (A,G,U)	UCG
1	1.62	0.40	1.64	0.39	0.43
2	0.32	0.10	0.37	0.13	0.12
3 + 4	0.72	0.14	0.39	0.18	0.18
5 + 6	3.52	1.71	2.59	2.53	2.43
7	5.68	1.08	3.46	1.26	1.15

[a] Assays contained 15 nmol of polyribonucleotides (in base residues) or 2.5 nmol of trinucleotide. Other details are described in Materials and Methods.

tions used (Fig. 8), and we found that there was no significant difference in the coding response between the two seryl-tRNA populations tested (Table 5).

DISCUSSION

The present study performed with T5st0 phage, a deletion mutant for several tRNA genes as announced by Chen et al. (Fed. Proc. **33**:1280, 1974), provides evidence that genes for serine, isoleucine, methionine, tyrosine, valine, and arginine tRNA's are located on the H strand. The localization of these tRNA genes is in agreement with the findings of Chen et al. (Fed. Proc. **33**:1280, 1974).

We did not test other tRNA's but we further examined hybridization of the seryl-tRNA which gave the highest hybridization value to obtain quantitative results. We found that two molecules of seryl-tRNA hybridized per T5st0 H strand. Seryl-tRNA from infected bacteria was analyzed by reversed-phase chromatography, hybridization experiments with T5st0 or *E. coli* DNA, and competition hybridization experiments with T5st0 DNA. We demonstrated the existence of two T5-specific isoacceptor serine tRNA species possessing distinctive nucleotide sequences and therefore transcribed from different sites on T5st0 DNA. Recently evidence has been reported (4) that T5 bacteriophage codes for the synthesis of two "isoacceptor" methionine tRNA species; however, these two species are tRNA$_f^{Met}$ and tRNA$_m^{Met}$, which perform different functions in protein synthesis. Moreover, we found that one T5-specific tRNA^{Ser} possesses various chromatographic behaviors which may depend on post-transcriptional modifications and also on conformational changes which should occur during the isolation procedure. Since T5st0 phage has been shown to be partially deleted for T5tRNA genes (Chen et al., Fed. Proc. **33**:1280, 1974), we cannot exclude that other tRNA^{Ser} species might be coded by wild-type T5 phage.

Some variations in the reversed-phase chromatography elution patterns were observed since the relative proportions of the two additional peaks containing only T5-specific seryl-tRNA varied according to our seryl-tRNA prep-

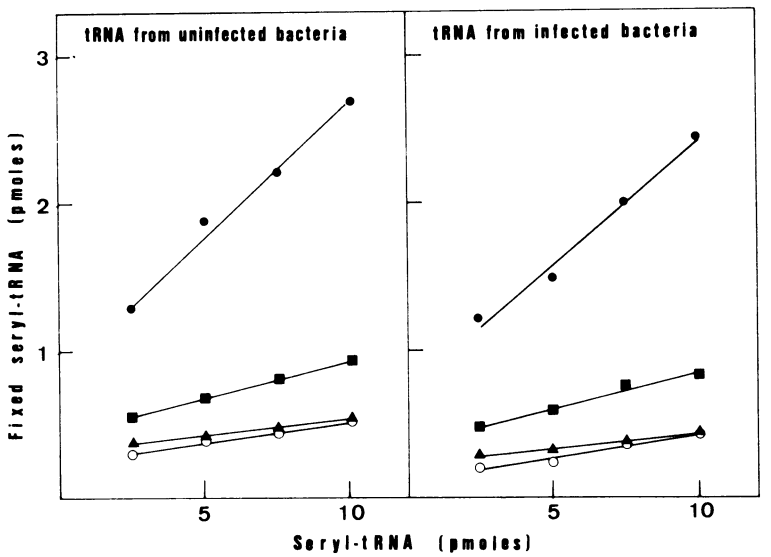

FIG. 8. *Binding of [^{3}H]seryl-tRNA from uninfected and infected bacteria to ribosomes as a function of the seryl-tRNA concentration in the presence of polyribonucleotides or trinucleotides. Each assay contained 15 nmol of polyribonucleotides (in base residues) or 5 nmol of trinucleotide. Binding minus template* (○), *plus poly(A,G,U)* (▲), *plus UCG* (■), *and plus poly(U,C)* (●). *Other details are described in Materials and Methods.*

TABLE 5. *Binding of [^{3}H]seryl-tRNA from uninfected and infected bacteria to ribosomes in the presence of polyribonucleotides or trinucleotides*[a]

Source of tRNA	Seryl-tRNA bound to ribosomes[b] (pmol)		
	Poly (U,C)	Poly (A,G,U)	UCG
Uninfected bacteria	2.17	0.02	0.43
Infected bacteria	2.11	0.01	0.39

[a] Data, from Fig. 8, are expressed for 10 pmol of seryl-tRNA.

[b] Corrected from the assay minus template.

arations. This may be a consequence of the conditions of incubation of the infected bacteria (infection not synchronized, arrest of infection by chilling), from uncontrolled factors during the tRNA isolation procedure, or from incomplete tRNA aminoacylation or from deacylation which could occur with different rates according to the seryl-tRNA species.

We provided evidence that the two T5-coded tRNA^{Ser} species recognize UCC or UCU codons previously assigned for serine. The presence of these two T5-coded tRNA^{Ser} species does not change the coding response of the seryl-tRNA. However, the T5-coded tRNA^{Ser} species might enhance the coding response of the seryl-RNA for poly(U,C) since they accounted for an important part of the total seryl-tRNA for the tested preparation from infected bacteria. Indeed, according to the RPC5 elution pattern, the T5-coded tRNA^{Ser} species contained in the two additional discrete peaks accounted for about 20% of the total seryl-tRNA, but a probably important part of T5-coded tRNA^{Ser} is contained in other peaks and cannot be directly estimated. As a possible explanation, we might suppose that the appearance of the two T5-coded tRNA^{Ser} species would be counterbalanced by the disappearance or the inactivation of *E. coli* tRNA^{Ser} species recognizing the same codons. However, the techniques used are not accurate enough to make definite conclusions. Nevertheless, these two T5-coded tRNA^{Ser} species recognize codons which are the most recognized by the *E. coli* tRNA^{Ser}. What is the function of these two T5-coded tRNA^{Ser} species if they are employed in protein synthesis? Do they increase the reading capacity for codons readable by the main part of *E. coli* tRNA^{Ser}? Can we compare the function of these T5-coded tRNA^{Ser} species with the likely function of the T4-coded tRNA's? In vitro triplet-binding experiments and studies of protein synthesis directed by T4 or *E. coli* mRNA's with T4-coded tRNA's (19), examination of burst size, and in vivo protein synthesis with tRNA-deficient T4 strains (24) suggested that T4-coded tRNA's ensure optimum rates of pro-

tein synthesis by supplementing the reading capacity for codons less used in *E. coli*. Studies were also performed with several *E. coli* strains other than the commonly used ones (25) and suggested that, during the evolution of T4 bacteriophage, synthesis of tRNA's was necessary to allow phage development in host, including tRNA's not adjusted to translate phage messengers. During the T5 development the synthesis of two phage-coded tRNA^{Ser} species does not seem to modify the reading capacity for codons less used in *E. coli* F; however, this synthesis may be necessary in other hosts.

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