EVIDENCE FOR THE EARLY SYNTHESIS OF T4 BACTERIOPHAGE-CODED TRANSFER RNA

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Summary: Synthesis of T4-coded tRNAs is an early phage function as detected by hybridization to the separated strands of T4 DNA and by hybridization competition experiments with radioactive T4 tRNA. When T4 infection is carried out in the presence of chloramphenicol, T4 tRNAs are transcribed which are capable of amino acylation, in vitro. These results are discussed.

Several laboratories have now reported the synthesis of viral-coded tRNAs after T-even phage infection of Escherichia coli[1-3]. More recently, Scherberg and Weiss[4] have found that bacteriophage T5 also codes for the synthesis of its own tRNAs.

^{35}S-tRNA from pulse-labeled T4-infected cells show 3 peaks of radioactivity on methylated albumin kieselguhr (MAK) chromatography whereas only 2 major ^{35}S-tRNA peaks are seen with uninfected E. coli[5]. The third ^{35}S-peak represents (in part) the de novo synthesis of T4-specific tRNA since 25 to 30% of the labeled RNA is hybridizable to T4 DNA, and both leucine and proline T4-specific tRNAs are present in this same peak fraction[1]. This report describes results from studies using "third peak" T4 ^{35}S-labeled tRNA, as well as T4 (^{3}H)leucyl-tRNA, which indicate that phage tRNA synthesis is an early function of the replicative process and suggests, in addition, that this synthesis is independent of viral-induced protein formation.

Results and Discussion: Earlier observations on the timing of "peak 3" appearance after phage infection indicated that T4 ^{35}S-tRNA formation is probably an early viral function[5]. This conclusion is now more firmly supported by the following experiments. Nonradioactive RNA was prepared from cells infected with T4 phage in the presence of chloramphenicol (CAP) and incubated at 37° for various time intervals (0-60 min) after infection. The RNA extracted from CAP-blocked, T4-infected cells was tested for its ability to compete with T4 "peak 3" ^{35}S-tRNA for hybridization to T4 DNA. Figure 1 shows that despite pre-infection addition

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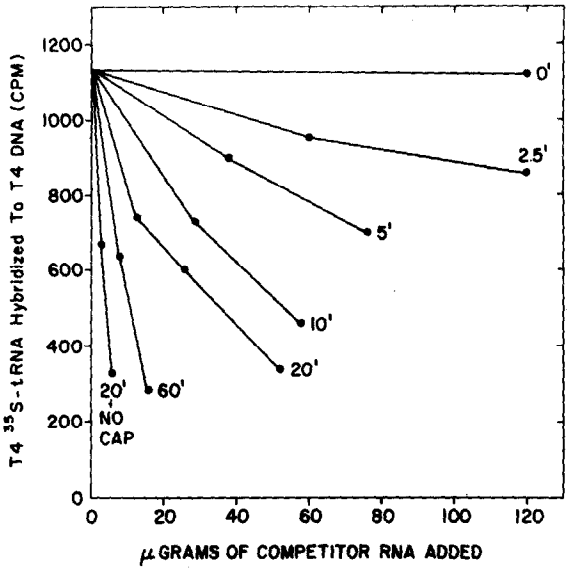

Footnote to Figure 1. Hybridization of T4 ^{35}S-tRNA to T4 DNA in the presence of competing RNA isolated from CAP-blocked cells at different times after T4 infection.

The annealing mixture (0.60 ml) contained 60 μg of denatured T4 DNA fixed onto nitrocellulose filters, 0.66 μg of T4 "peak 3" ^{35}S-tRNA (1.1×10^4 cpm per μg), various amounts of competitor RNA as indicated above, 0.3 M NaCl - 0.03 M citrate pH 5.5, and 0.30 ml of formamide. After annealing for 20 hours at 37°, the filters were washed and RNase treated as previously described[1], and the radioactivity "fixed" onto the filters determined by scintillation counting. The preparation of T4 ^{35}S-tRNA (MAK peak 3) and the conditions for E. coli growth and T4 phage infection were as reported elsewhere[1]. Competitor RNA was extracted from E. coli B infected with T4 phage for 20 min. in the absence of CAP, and for 0, 2.5, 5, 10, 20 and 60 min. in the presence of CAP (50 μg per ml of culture) which was added to the culture medium 2 min. prior to infection. The stopping of infection at the time intervals indicated, their collection and the isolation of RNA was as previously reported[5].

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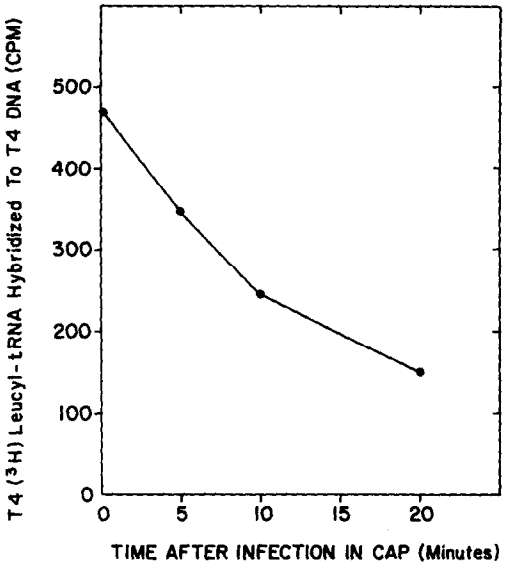
of CAP, RNA isolated as early as 2.5 minutes after infection competes with T4 ^{35}S-tRNA for hybridization sites on T4 DNA. With increasing times of infection the infected-cell RNA competes more effectively with the labeled T4 tRNA in the hybridization assay, suggesting the accumulation of RNA homologous to viral tRNA. On a weight basis, the rate of accumulation of viral tRNA competitor is slower in the presence of CAP than in its absence (compare the 20 minute infected RNA preparations in Figure 1). This difference may reflect a slower rate of viral RNA transcription in the presence of CAP or it may simply represent a lower percentage of viral tRNA competitor per μg of total RNA extracted, since, in the presence of CAP, host RNA synthesis is not shut off after phage infection[6-9].

In agreement with the data presented above, Figure 2 shows that the tRNA



Footnote to Figure 2. Time of RNA appearance in T4-infected CAP-blocked cells which competes with T4 (^{3}H)leucyl-tRNA for hybridization to T4 DNA.

The annealing mixture was the same as described for Figure 1 except that the radioactive nucleic acid used was T4 (^{3}H)leucyl-tRNA (12.8 µg containing 108,800 cpm of (^{3}H)leucine with a specific activity of 58 c/mM), filters contained 39 µg of denatured T4 DNA, and 68 µg of competitor RNA isolated from cells infected with T4 phage in the presence of CAP (50 µg per ml of culture) for 0, 5, 10 and 20 minutes was individually added as indicated above. After annealing for 4 hours at 35° the extent of (^{3}H)leucyl-tRNA hybridization to the T4 DNA-filters was determined as described for Figure 1.

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isolated from E. coli cells infected with T4 phage in the presence of CAP also competes with T4 (^{3}H)leucyl-tRNA for hybridization sites on T4 DNA. Once again, significant competition occurs with RNA isolated early after infection, and the capacity for the isolated RNA to compete with viral tRNA increases with longer periods of infection.

Additional evidence suggesting that viral tRNA is an early function of vegetative phage development was obtained from the hybridization characteristics of ^{35}S-labeled T4 tRNA to the separated *l* and *r* strands of T4 DNA[10]. Table 1 shows that when annealing to the respective strands of T4 DNA was carried out, 25% of the input counts hybridized to the *l* strand, whereas the radioactivity fixed to the *r* strand was at the background level. Since the early classes of T4 phage RNA are transcribed from the *l* strand[10], this result is consistent with the conclusion that phage tRNA transcription is an early event in the phage maturation process.

The presence of RNA molecules capable of competing with acylated phage tRNAs (Figure 2) in CAP-blocked infected cells, led us to enquire whether the

Table 1

Hybridization Pattern of T4 ^{35}S-tRNA with the Separated Strands of T4 DNA

DNA	Input Radioactivity (c.p.m.) a	c.p.m. annealed	c.p.m. annealed corrected for blank filter b	% hybridized $\frac{b}{a} \times 100$	Percent $\frac{\ell}{r}$
T4 ℓ strand	4436	1137	1003	25	100
T4 r strand	"	131	0	0	0
λ dN DNA	"	190	56	1.3	-
E. coli dN DNA	"	198	64	1.4	-
Blank Filter (-DNA)	"	134	-	-	-

Footnote to Table 1

T4 ^{35}S-tRNA collected from Peak 3 fraction of the MAK column was isolated from T4 infected E. coli B cells following the technique described by Weiss et al.[1] The separation of the complementary T4 DNA strands with poly U,G was as described by Guha and Szybalski[10]. The hybridization technique of Nygaard and Hall[14] was essentially followed.

Table 2

The Annealing of Charged tRNAs Isolated from Chloramphenicol T4-Infected Cells, with T4 DNA

Amino Acid	Specific Activity	Charged T4 tRNA	DNA fixed on Filter		Competitor E. coli tRNA	Radioactivity fixed on filter
			T4	T5		
	c/mM	µg (c.p.m.)				(c.p.m.)
Arg	45	24 (632,000)	+	-	-	551
		" "	+	-	+	555
		" "	-	+	-	186
Gly	20	24 (536,000)	+	-	-	1187
		" "	+	-	+	1160
		" "	-	+	-	94
Leu	58	24 (158,000)	+	-	-	330
		" "	+	-	+	293
		" "	-	+	-	55

Footnote to Table 2

The conditions for annealing were as described for Figure 2. RNA was isolated from cells infected for 20 min. with T4 phage in the presence of chloramphenicol (100 µg per ml), purified and charged with the (^{3}H) amino acids shown as previously reported[1]. The amount of charged tRNA used is shown. The quantities of T4 and T5 DNA impregnated onto filters were 45 µg and 35 µg, respectively. Where shown, 150 µg of E. coli tRNA was included in the annealing mixture. Hybridization of (^{3}H) aminoacyl-tRNA was determined as described for Figure 1. Five T4 tRNA species have been identified which include arg, gly, leu, ileu and pro[4].

competitor RNA itself was functional with respect to amino acid charging. Table 2 shows that T4 phage-specific tRNAs capable of accepting arginine, glycine, and leucine (proline and isoleucine were not tested) are formed in CAP-blocked T4-infected cells. These results suggest the following possibilities: 1) Phage tRNAs do not require chemical modification (i.e., methylation, thiolation, pseudouridine formation, etc.) for amino acid charging; 2) Phage tRNAs do require chemical modification for amino acid acceptor activity, but host modifying enzymes, and not viral-induced enzymes, are employed for this function.

Several comments should be made in considering these two possibilities. Although the base composition of viral-tRNAs is unknown, several independent observations suggest that T4 tRNAs include methylated[11] and thiolated bases[1,5] as well as pseudouridine[12]. Furthermore, the synthesis of phage-induced tRNA modifying enzymes in infected-cell preparations is indicated by the following: 1) Discrete changes in the relative activities of the different base-specific tRNA methylases[13] as well as changes in rates of tRNA sulfuration[5] have been

observed after T-even phage infection of E. coli. 2) In the presence of chloramphenicol, T4 ^{35}S-tRNA (MAK, peak 3) formation does not occur, suggesting that some viral-induced enzyme is responsible for T4 tRNA thiolation[5]. These findings, including those reported in this paper, raise the possibility that when infection is carried out under conditions where protein synthesis is inhibited, T4 tRNAs are transcribed but not modified with respect to the formation of certain minor bases. If this interpretation is correct, then the fact that such tRNA molecules can be aminoacylated would indicate that these minor bases play no obvious role in the charging of viral tRNAs.

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