

Changes in Leucine-Specific sRNA after Infection of *E. coli* by Phages T2 and T4

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ABSTRACT Normal development of T2 and T4 in *E. coli* can occur without major alterations of the leucyl-specific sRNA contingent of the bacterium. The minor changes observed are apparently bacterial strain-specific. They show no temporal relation to the shift from early to late function in normal phage development.

INTRODUCTION

Sueoka et al. (1) examined changes in the sRNA of *E. coli* B before and after infection by phage T2. Among sRNAs for seventeen amino acids examined, only leucine-specific sRNA showed appreciable differences. One possible interpretation was that the observed alterations are required for normal phage development, an interpretation with important implications concerning the regulation of synthesis of phage-specific proteins. The results reported here demonstrate that under the analytical conditions employed in this study, the pattern of changes observed by Sueoka et al. does not occur in other strains of *E. coli* infected with either T2 or T4; yet these strains can support normal phage development.

MATERIALS AND METHODS

Strains *E. coli* B was obtained from S. Benzer, *E. coli* W1485 (λ^-) from E. Lederberg, and T2 and T4 wild-type were obtained from S. E. Luria.

Media Standard medium (SM) was 8 g Bacto-tryptone, 5 g NaCl in 1000 ml glass-distilled H₂O.

Plates Bottom agar, SM + 1.5% Bacto-agar; top layer, SM + 0.7% bacto-agar.

Conditions of Phage infection *E. coli* strains were maintained on SM slants. Cultures were begun by inoculating 100 ml of SM medium from a slant. After growth overnight with shaking at 37°C, the entire stationary culture was used as an inoculum for 1000 ml of aerated, prewarmed SM broth. Doubling time followed on a Klett turbidimeter was 30 to 35 min at 37°C. When the culture reached 4×10^8 viable

cells/ml, T2 or T4 (10^{11} phages/ml, concentrated by centrifugation from DNAase-treated lysates) were added in 100 ml of prewarmed SM at a multiplicity of 5. Less than 5% of the bacteria were still viable at 3 min after infection. At 5 and 10 min after infection 500 ml of the culture were instantly chilled to 4°C by pouring over 2 volumes of packed, crushed ice. Cells were pelleted at 10,000 *g* for 10 min in the cold and stored at -15°C. When infection was allowed to proceed, total lysis occurred by 45 min. Recovery after purification of the lysate was 50 viable phage/cell.

Preparation of sRNA from Infected Cells sRNA was extracted from the infected cells by the method of von Ehrenstein and Lipmann (2) with the following modifications. The final alcohol precipitate was taken up directly in 1 ml of 0.5 M Tris-HCl,

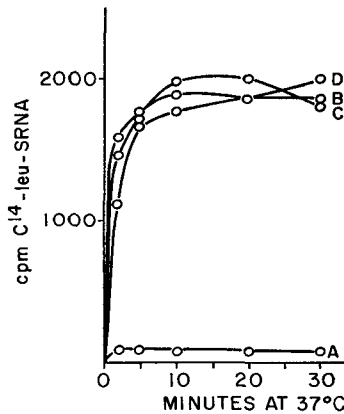


FIGURE 1. Enzymatic charging of sRNA. Various amounts of a crude activating enzyme preparation obtained from uninfected *E. coli* B were incubated in 1 ml at 37°C with 0.26 mg of sRNA from *E. coli* B. 0.1 ml aliquots were precipitated in 10% trichloroacetic acid and counted at 70% efficiency. (A), no enzyme; (B), 0.17 mg enzyme protein; (C), 0.43 mg; (D), 0.85 mg. Specific activity at plateau is approximately 9×10^4 DPM/mg sRNA. Standard reaction mixture used in subsequent experiments contained 0.17 mg enzyme protein. At plateau one sRNA molecule in 160 was bonded to leucine.

pH 8.8, and incubated for 45 min at 35°C to remove any attached amino acids. The sRNA was then immediately cooled, passed over Sephadex (G-25 fine, equilibrated with 10^{-2} M Tris-HCl, pH 7.3, plus 10^{-3} M $MgCl_2$, 1 \times 10 cm) to remove small molecules and return the pH to 7.3, and lyophilized to obtain a final concentration of about 1 mg/ml. The yield of discharged sRNA was between 0.5 and 1.0 mg/ 2×10^{11} bacteria.

Amino-Acyl-sRNA Synthetase The enzyme was prepared by the method of Yamane and Sueoka (3). It was freed of RNA (see below) by passage over a DEAE-cellulose column.

C^{14} -Leucyl-sRNA Discharged sRNA was charged in a reaction mixture containing, per milliliter, 125 μ moles Tris-HCl, pH 7.3; 1.25 μ moles ATP; 12.5 μ moles $MgCl_2$; 12.5 μ moles KCl; 5 μ moles reduced glutathione; 2 μ moles each of seventeen

cold amino acids; C^{14} -L-leucine (201 mc/mm, New England Nuclear Corp.); 0.17 μ g enzyme protein and 0 to 0.5 mg sRNA. At 37°C the charging reaction was complete by 10 min and the plateau was maintained for at least 30 min. In experiments to be described below, 0.2 to 0.3 mg sRNA from *E. coli* was charged in 1 ml of this reaction mixture. At this concentration, the enzyme is in excess (Fig. 1). No sRNA contaminated the enzyme preparation (Table I). There was a low level of counts seen in the absence of added sRNA which did not increase after 2 min of incubation and was wholly resistant to RNAase. These counts may have represented trapped leucyl-adenylate enzyme (4).

TABLE I
TEST OF ENZYME PREPARATION FOR CONTAMINATING sRNA

Time at 37° C	CPM C^{14} leucine/0.17 mg enzyme protien			
	No added sRNA		1.0 mg/ml sRNA	
<i>min</i>				
2	147		335	
5	183		1468	
10	130		1676	
	At 11 min, reaction mixture transferred to buffer with (a) or without (b) RNAase			
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
13	104	112	122	1822
16	155	224	162	1690
27	155	114	170	1595

Standard reaction mixtures were incubated at 37°C in the presence and absence of added sRNA. 0.1 ml aliquots were precipitated in 1 ml of cold 10% trichloroacetic acid at 2, 5, and 10 min of incubation. At 11 min the remaining reaction mixtures were divided and diluted tenfold into prewarmed buffer containing either 0 or 15 μ g bovine pancreatic ribonuclease per ml. At 13, 16, and 27 min total incubation time, 1 ml aliquots were precipitated in cold trichloroacetic acid at a final concentration of 10%. With 0.4 mg crude DNA as carrier, all precipitates were trapped on Millipore membrane filters and washed 3 times in a total of 50 ml of cold 10% trichloroacetic acid. The filters were dried and suspended in toluene base scintillation fluid (Liquifluor, New England Nuclear Corp.) and counted to 1000 counts at 70% efficiency. Background (45 cpm) was not deducted. Final specific activity in the absence of RNAase was about 25,000 DPM/MG, due to the excess amount of sRNA present in this experiment.

MAK Chromatography After a 15 min incubation charged sRNA was isolated from the reaction mixture by extraction on ice three times with water-saturated phenol (Mallinckrodt) and twice with ether. sRNA was precipitated with two volumes of ethanol at -15°C for 1 hr, suspended in 50 ml of 0.05 M sodium phosphate, pH 6.3, plus 0.2 M NaCl, and fractionated on a four layer MAK column (5) eluted according to the methods of Sueoka et al. (1, 3). Optical density at 260 μ was monitored quantitatively in a Gilford-Beckman continuous flow spectrophotometer. After each gradient, the column was washed with 500 ml of the initial buffer and stored at 5°C. A single MAK column was used eight times this way with no loss of resolution. NaCl concentration of eluted 2 ml fractions was determined with a previously calibrated Abbe refractometer. Charged sRNA was precipitated from the 2 ml fractions by

addition of cold trichloroacetic acid to a final concentration of 10%. With 0.4 mg crude DNA as carrier, precipitates were trapped on Millipore membrane filters, washed twice with cold 10% trichloroacetic acid, and counted at 70% efficiency to 1000 counts in a Nuclear-Chicago Corp. scintillation counter.

RESULTS

Preparation of C¹⁴-leucyl-sRNA *E. coli* strains B and W were synchronously infected with bacteriophages T2 or T4. sRNA was isolated from cultures at 0 time and at 5 and 10 min after infection. Amino acids were removed by the mild high pH treatment and the sRNA was recharged in a reaction mixture containing C¹⁴-leucine and seventeen cold amino acids (as described in the Methods section). sRNA was reextracted from the reaction mixtures after 15 min incubation and stored at -15°C for chromatography on a MAK column.

MAK Column Chromatography Charged sRNA, including C¹⁴-leucyl-sRNA, was eluted by a linear NaCl gradient from a methylated albumin-kieselguhr column. The resulting profiles of leucyl-sRNA for the T2-*E. coli* B and T2-*E. coli* W series are shown in Fig. 2. T4-infected cells produced the same results.

The MAK column split leucyl-sRNA from uninfected *E. coli* into two major peaks. An additional small separate peak was found before infection in *E. coli* W but not in *E. coli* B (Fig. 2 A, D). This small peak was also detected in both strains at 5 min after infection (Fig. 2 B, E). It was still present at 10 min after infection (Fig. 2 C, F). A minor shoulder to the first major peak appeared at 5 min in both strains and was still present at 10 min after infection. The relative heights of the two major peaks did not change in B and W after phage infection.

DISCUSSION

These results differ from previous studies on the T2-*E. coli* B¹ system at three points. Sueoka et al. (1) found (a) a large drop in the first major peak of leucyl-sRNA by 5 min after infection. This peak remained low until 8 min, the latest time observed; (b) a minor peak comparable to the one reported here but which was detectable only from 3 to 5 min after infection, and which was gone by 8 min; (c) no resolvable shoulder to the first main peak.

The evaluation of these data depends on two crucial requirements: (a) that the reproducibility of successive runs on a single MAK column be high enough to permit comparisons; and (b) that the resolving power be great

¹The strain of *E. coli* employed in these experiments was not obtained from Dr. Sueoka and is undoubtedly not isogenic with his strain.

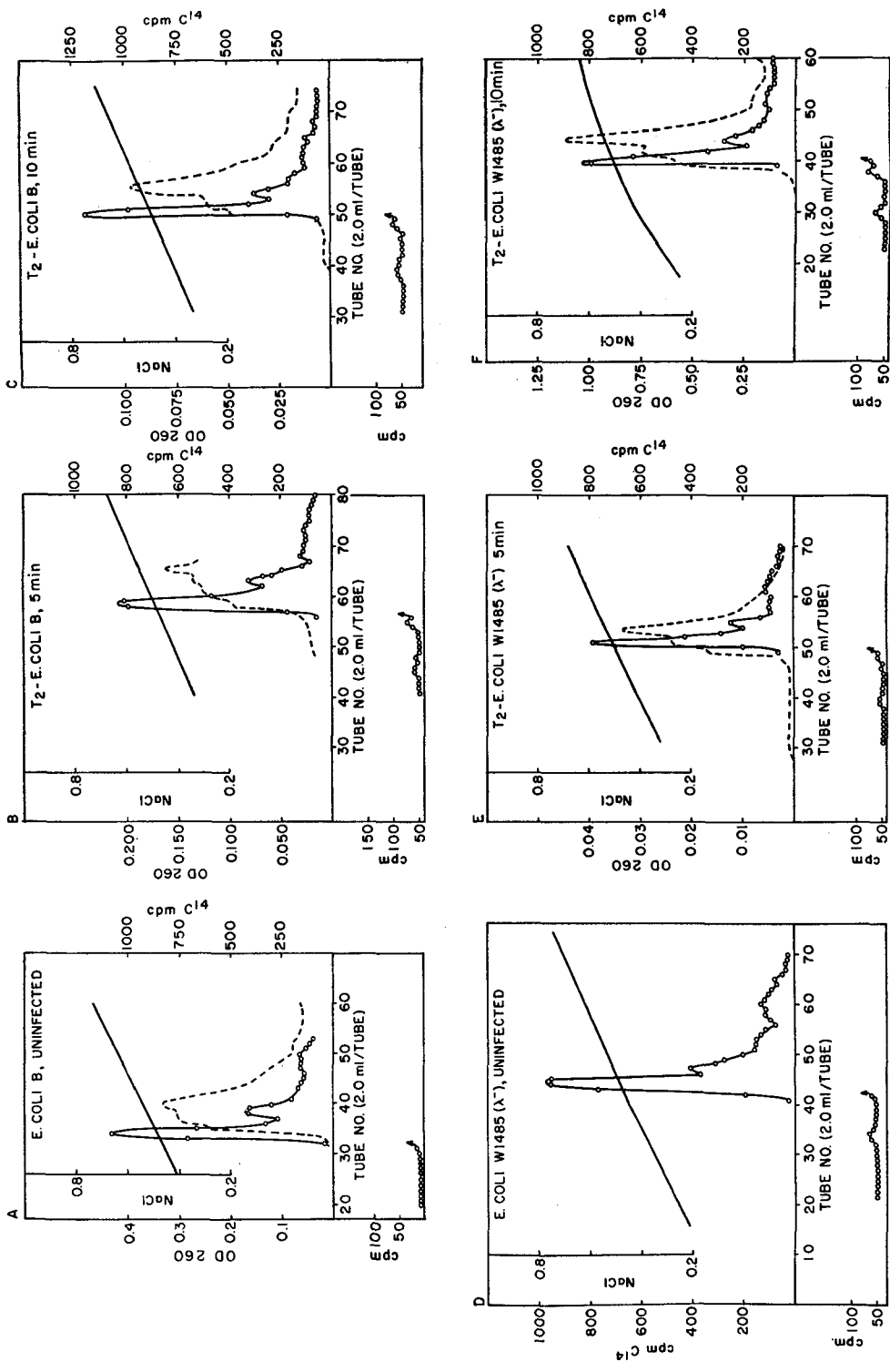


FIGURE 2. Leucyl-sRNA at different times after T2 infection. The RNA was taken from uninfected cells and cells infected for 5 and 10 min and chased by *E. coli* B enzyme free from RNA contamination. (A) *E. coli* B, uninfected; (B) *E. coli* B T2, 5 min; (C) *E. coli* B T2, 10 min; (D) *E. coli* W, uninfected; (E) *E. coli* W T2, 5 min; (F) *E. coli* W T2, 10 min. Fig. 2A is taken from a double label experiment, all other experiments contained only C^{14} and were counted at 70% efficiency.

enough to distinguish dependably between the two closely juxtaposed major peaks.

The first is particularly important since differently labeled marker sRNA was not employed as an internal control as was done in the earlier study (1). Table II summarizes the NaCl concentrations at which the different peaks were eluted in the experiment illustrated in Fig. 2. They show that the difference in salt concentration required to elute the two main peaks in any one run is considerably larger than the variation in salt concentration for either peak in six successive runs. Furthermore, an additional set of four experiments performed with B and W-1485 infected with phage T4 yielded the same level of precision. It should be noted that this degree of reproducibility cannot be

TABLE II
NaCl CONCENTRATIONS REQUIRED TO ELUTE SPECIES OF LEUCYL-sRNA

Run (Fig. 2)	NaCl concentration, M		Distance between major peaks			
	Minor peak	Shoulder	Major peaks		$\Delta(\text{NaCl})$	Δ ml of eluate
			1	2		
A	None	None	0.49	0.53	0.040	9
B	0.40	0.46	0.49	0.53	0.040	8
C	0.40	0.47	0.495	0.53	0.045	8
D	0.38	None	0.49	0.51	0.020	6
E	0.41	0.49	0.50	0.54	0.040	8
F	0.41	0.48	0.50	0.54	0.040	8
Mean	0.400	0.475	0.494	0.530	0.038	7.83
Standard deviation	± 0.012	± 0.011	± 0.005	± 0.010	± 0.008	± 0.28

The MAK column elutions shown in Fig. 2 provided the data for this table.

attained when different MAK columns are employed and in these an internal control is indeed necessary.

The second requirement also seems to have been met. As may be seen from Table II, the two major peaks were separated by 7.8 ± 0.3 ml (about 4 fractions) in the eluates. This separation and the half-width of OD_{260} profiles are comparable to the ones reported previously by Sueoka. Resolution within comparable sets of gradients was at least as high in these experiments as in the earlier report, as judged from (a) the narrowness of the main leucyl-sRNA peaks, (b) the resolution of a shoulder to the first main peak, and (c) the reproducible, complex total sRNA OD_{260} profile.

The results reported here indicate that neither the appearance and disappearance of a minor species of leucyl-sRNA nor alterations in the major peaks as previously reported do in fact represent mandatory contributions by the phage genome to normal phage development. The existence of the minor shoulder though interesting cannot be evaluated at this time. In principle its

relevance to phage development might be assessed by comparison with phage infection initiated by amber mutants (6, 7) lacking early functions.

Note Subsequent work by Sueoka and Kano-Sueoka (8) brought to the attention of this author after submission of this manuscript, has shown that the timing of the modifications of leucyl-sRNA observed by them is variable and that under conditions of uniform and efficient phage infection the modifications could be completed in an extremely early stage of infection. They concurred that the involvement of the modification in the transition of the early to the later phase of protein synthesis is unlikely.

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