A PHYSIOLOGICAL ROLE FOR tRNA NUCLEOTIDYLTRANSFERASE DURING BACTERIOPHAGE INFECTION[1]

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SUMMARY: Bacteriophage infection of E. coli cells deficient in the enzyme tRNA nucleotidyltransferase (cca mutants) resulted in greatly decreased production of viable progeny phage compared to wild type cells. This decrease amounted to as much as 90% in the case of T-even bacteriophages, and 50-65% for T-odd bacteriophages. However, infection by the RNA phages, Qβ and f2, was unaffected by the cca mutation. Examination of T4 infection of cca hosts indicated that phage development proceeded normally, that near-normal numbers of progeny particles were formed, but that most of these particles were nonviable. Possible functions for E. coli tRNA nucleotidyltransferase during bacteriophage infection are discussed.

INTRODUCTION: In vitro tRNA nucleotidyltransferase (E.C.2.7.7.25) catalyzes the synthesis of the -C-C-A terminus of tRNA (1). However, the function of this enzyme in vivo has remained unclear. Earlier studies with Escherichia coli strains (cca mutants) deficient in this enzyme (2) revealed that tRNA nucleotidyltransferase was required for normal cell growth (3), and for repair of tRNA molecules with incomplete 3' terminal sequences (2). In addition, tRNA nucleotidyltransferase appears to be required for the biosynthesis of a small number of E. coli tRNAs (unpublished observations).

During infection of E. coli by bacteriophage T4, eight phage-specific tRNAs are synthesized (4), and host tRNA nucleotidyltransferase is involved in the processing of several of these molecules (3,5). Despite the fact that these phage-specified tRNAs are not required for successful T4 infection (6), we observed that the burst size of this bacteriophage was greatly decreased in all cca⁻ hosts (2). Since these results suggested that tRNA nucleotidyltransferase might have an additional function during phage infection, we have

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TABLE I

Burst size of bacteriophage T4 in various *cca*$^+$, *cca* and revertant strains

Strain	Burst size	
	cca$^+$	*cca*
A19	74	9
KL14	95	5
MD100	84	8
JF172	95	17
JF172 revertants (6)	71 - 109	

Cells were grown to 2 x 10^8 cells per ml and infected at a multiplicity of about 0.01 in YT medium in the presence of KCN (5 mM) and tryptophan (25 µg/ml). After 10 min at 37° the infected cells were diluted by 10^3 and shaken at 37°. Samples were taken at various times for determination of phage titer. The results presented were calculated from the titer of phage at the plateau divided by the number of infecting phage particles. Controls with chloroform were performed to ensure complete release of phage at the plateau, and for measurement of unadsorbed phage.

examined this process in more detail. Our initial data, reported here, indicate that tRNA nucleotidyltransferase is required for normal infection by a variety of DNA-containing bacteriophages, and that the absence of this enzyme leads to the production of non-viable phage particles.

MATERIALS AND METHODS: *E. coli* strains A19, KL14 and JF172 have been described earlier (3,7). Strain MD100 (*pro*, *trp*, *thi*, *ade*, *ura*) was obtained from Dr. Richard Berlin. All the *cca* strains used in this study were prepared from strains 35-10 (2) using phage P1-mediated transduction and selection of *tolC*$^+$ transductants (7). JF172 *cca*$^+$ revertants were selected as described earlier (3). The various bacteriophage strains used in this study were wild type and were obtained from Drs. William McClain or Joseph Speyer. Cells were grown and infected in YT medium (8 g tryptone, 5 g yeast extract, 5 g NaCl per liter).

RESULTS: Previous studies indicated that each of five independently-isolated *cca* mutants could not support the normal growth of bacteriophage T4 (2). Since these strains were isolated after nitrosoguanidine mutagenesis, the possibility existed that the observed phenotype was not due to the *cca* locus, but to another mutation. However, as shown in Table I, the property of lowered

burst size could be transduced with the cca mutation into a variety of different genetic backgrounds, suggesting that the cca locus was responsible for the effect. This was shown conclusively by the isolation of single-step cca revertants. The revertants were isolated by their normal growth properties, which contrasted with the slow growth of the cca mutants. Each of six such revertants, which had simultaneously re-acquired normal tRNA nucleotidyltransferase activity (3), supported a typical infection by phage T4. These data demonstrate that the lowered burst was a consequence of the mutation at the cca locus.

Decreased bursts were also obtained when mutant cells were infected at a high multiplicity (m.o.i. = 5), or when the infection was carried out in defined media. Treatment of infected cells with chloroform late in infection indicated that the lowered bursts were not due to the incomplete release of mature phage. Furthermore, bacteriophage T4 plated with 100% efficiency on cca strains, although the plaque size was slightly smaller than on cca^+ strains.

Mutation at the cca locus also influenced infection by other bacteriophages. The data in Table II indicate that the other T-even phages, T2 and T6, were affected similarly to T4, with burst sizes decreased about 90%. The burst size of bacteriophages T5 and T7 were somewhat less sensitive to the cca mutation, being decreased about 65% and 50%, respectively. In each case, however, reversion at the cca locus restored the phage burst size to normal. Induction of bacteriophage lambda in the lysogenic strains A19 (cca^+) and 35-10 (cca) revealed a small, but reproducible, decrease in phage production under these conditions, as well. We have not isolated a revertant in the 35-10 background to conclusively show that the lowered level of lambda was a consequence of the cca mutation. Nevertheless, these data indicate that a variety of DNA-containing bacteriophages are affected by a cca mutation in the host. In contrast to the foregoing results, infection by the RNA bacteriophages, f2 and Qβ, was unaffected by the mutation in tRNA nucleotidyltransferase. These studies suggest that tRNA nucleotidyltransferase plays a role in a process specific to DNA bacteriophages.

TABLE II

Burst size of various bacteriophages in *cca*$^+$, *cca* and revertant strains

Bacteriophage	Burst size		
	cca$^+$	*cca*	*cca*$^+$ revertant
T2	122	5	120
T4	95	17	90
T6	47	5	51
T5	845	271	818
T7	139	71	136
λ (induction)	26	19	-
f2	7400	7600	-
Qβ	7500	7600	-

The burst sizes of the T bacteriophages were measured as described in Table I using *E. coli* strains of the JF172 background. Induction of bacteriophage lambda was accomplished by addition of 5 μg per ml of mitomycin C to *cca*$^+$ and *cca* strains of the A19 background. Eighty to ninety percent of the cells were induced by this procedure. The burst size was calculated from the maximum phage titer divided by the number of induced cells. The burst sizes of the RNA phages, f2 and Qβ, were measured on the Hfr *E. coli* strain KL14. Cells were infected at a multiplicity of 10, and excess phage were removed by centrifugation and washing. The burst size was calculated from the maximum phage titer divided by the number of infective centers.

In order to understand the reason for the lowered burst size of DNA bacteriophages in *cca* hosts, the time course and progeny of T4 infection of *cca*$^+$ and *cca* strains were examined in more detail. We found no differences in the course of phage development in *cca*$^+$ and *cca* hosts with regard to macromolecular synthesis, induction of early enzymes or lysozyme, or in the levels of defective tRNA (data not shown). Furthermore, the results in Table III demonstrate that the bacteriophage particle yield, as measured by absorbance, DNase-resistant radioactivity from a thymidine precursor, and killer particles, was the same from the wild type and mutant hosts. In marked contrast,

TABLE III

Characterization of progeny from bacteriophage T4 infection of *cca*$^+$ and *cca* strains

Properties	*cca*$^+$	*cca*
Absorbance at 260 nm	0.069	0.071
DNase-resistant thymidine cpm	1.71×10^5	1.66×10^5
Killer particles	9.7×10^9	6.9×10^9
Viable phage	6.7×10^9	5.9×10^8

E. coli strains A19 and 35-10 at 2×10^8 cells per ml were infected with T4 at a multiplicity of 5 in YT medium at 37°. After 10 min, [^{3}H] thymidine (20 μC, 10 μg per ml of culture) was added, and the infection allowed to proceed for another 45 min. The culture was treated with chloroform, and centrifuged to remove cell debris. The supernatant liquid was treated with pancreatic DNase (70 units per ml of culture) for 30 min at 37°, and centrifuged at 30,000 rpm for 60 min. The phage pellet was resuspended in M9 medium and recentrifuged. The final pellet was suspended in M9 medium. This purified phage preparation was used for the measurements presented. All data have been normalized to 1 ml of the phage preparation. Killer particles were determined by measurement of viable cells remaining after 10 min of infection, and calculation of the apparent multiplicity of infection from the Poisson distribution.

however, the number of viable bacteriophage produced in the *cca* host was only about 10% of that in the *cca*$^+$ strain. Although these measurements were carried out with partially purified progeny, identical results were obtained when crude lysates were examined, indicating that the purified particles are not a selected population. The data indicate that the lowered burst size of bacteriophage T4 in *cca* strains is due to the production of non-viable particles. We have not been able to separate the viable and non-viable progeny particles on the basis of either size or density (data not shown).

DISCUSSION: The results presented in this paper demonstrate that mutations at the *cca* locus in *E. coli* lead to a reduced burst size for a variety of DNA-containing bacteriophages. The fact that RNA phages were not affected suggests that a process specific to DNA-containing bacteriophages was involved,

although it should be pointed out that there is no direct evidence that the same step was affected with each phage. Our results with bacteriophage T4 indicate that infection of a cca host leads to the accumulation of non-viable virus particles. The reason for the non-viability of these particles is not yet clear, although preliminary results suggest that the defective phage can adsorb and inject their DNA normally.

Thus, the effect of the cca mutation does not become evident until the second cycle of infection. This suggests that the defect in the non-viable phage may reside in the viral DNA or in a protein, or other molecule, which is injected with the DNA. One possibility, which would be consistent with the known activity of tRNA nucleotidyltransferase, is that the enzyme is required for the proper functioning of the RNA molecule which is thought to be associated with the T4 genome (8). Such a molecule might participate in initiation of T4 DNA replication, for example, in a manner analagous to that occupied by tRNA in the reaction catalyzed by reverse transcriptase (9). If that were the case, synthesis of a functional 3' terminus on the primer might require the action of tRNA nucleotidyltransferase. Alternatively, the enzyme may serve a function during T4 infection unrelated to its known activity. For example, tRNA nucleotidyltransferase may act as a component of a phage protein injected with the viral DNA (10,11). Further studies will be required to differentiate among these, and other, possibilities.

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REFERENCES

1. Deutscher, M.P. (1973) Prog. Nucleic Acid Res. 13, 51-92.
2. Deutscher, M.P. and Hilderman, R.H. (1974) J. Bacteriol. 118, 621-627.
3. Deutscher, M.P., Foulds, J. and McClain, W.H. (1974) J. Biol. Chem. 249, 6696-6699.
4. McClain, W.H., Guthrie, C. and Barrell, B.G. (1972) Proc. Natl. Acad. Sci. 69, 3703-3707.
5. Guthrie, C., Seidman, J.G., Comer, M.M., Bock, R.M., Schmidt, F.J., Barrell, B.G. and McClain, W.H. (1974) Brookhaven Symposia in Biology 26, 106-123.

6. Wilson, J.H. (1973) J. Mol. Biol. 74, 753-757.
7. Foulds, J., Hilderman, R.H. and Deutscher, M.P. (1974) J. Bacteriol. 118, 628-632.
8. Speyer, J.F., Chao, J. and Chao, L. (1972) J. Virol. 10, 902-909.
9. Dahlberg, J.E., Harada, F. and Sawyer, R.C. (1974) Cold Spring Harbor Symposia on Quantitative Biology XXXIX, 925-932.
10. Horvitz, H.R. (1974) J. Mol. Biol. 90, 739-750.
11. Black, L.W. and Abremski, K. (1974) Virology 60, 180-191.
12. Deutscher, M.P. and Evans, J.A. (1976) Journal of Molecular Biology (In Press)