JOURNAL OF VIROLOGY, Aug. 1984, p. 522–529
0022-538X/84/080522-08$02.00/0

Vol. 51, No. 2

Genetic and Physiological Studies of an *Escherichia coli* Locus That Restricts Polynucleotide Kinase- and RNA Ligase-Deficient Mutants of Bacteriophage T4†

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Received 9 December 1983/Accepted 8 May 1984

The RNA ligase and polynucleotide kinase of bacteriophage T4 are nonessential enzymes in most laboratory *Escherichia coli* strains. However, T4 mutants which do not induce the enzymes are severely restricted in *E. coli* CTr5X, a strain derived from a clinical *E. coli* isolate. We have mapped the restricting locus in *E. coli* CTr5X and have transduced it into other *E. coli* strains. The restrictive locus seems to be a gene, or genes, unique to CTr5X or to be an altered form of a nonessential gene, since deleting the locus seems to cause loss of the phenotypes. In addition to restricting RNA ligase- and polynucleotide kinase-deficient T4, the locus also restricts bacteriophages λ and T4 with cytosine DNA. When λ or T4 with cytosine DNA infect strains with the *prr* locus, the phage DNA is injected, but phage genes are not expressed and the host cells survive. These phenotypes are unlike anything yet described for a phage-host interaction.

The RNA ligase and polynucleotide kinase of T4 are two of the most studied and useful of the enzymes induced by the bacteriophage. Their structural genes, genes 63 and *pseT*, respectively, are closely linked and lie between kilobases 130 and 155 on the T4 genome (22, 23, 32). Besides RNA ligase activity, the product of gene 63 has an apparently unrelated activity involved in tail fiber attachment (31). Thus, we shall refer to mutations in gene 63 which specifically inactivate RNA ligase as *rli*$^-$ mutations.

Both RNA ligase and polynucleotide kinase are nonessential for T4 development on most laboratory *Escherichia coli* strains. However, on clinically isolated *E. coli* CT196 and on a derivative of this strain, CTr5X, both *pseT*$^-$ and *rli*$^-$ mutants are severely restricted (10, 21). After infection of *E. coli* CTr5X, both *pseT*$^-$ and *rli*$^-$ mutants exhibit defects in T4 DNA replication and late gene expression (10, 21). Furthermore, both *rli*$^-$ and *pseT*$^-$ mutations share the same extracistronic suppressor, *stp* (10, 21, 22). This, as well as other evidence, has led us to propose that the RNA ligase and polynucleotide kinase are involved in the same reaction in vivo (21); but the nature of this reaction remains unknown.

To further study the function of the T4-induced polynucleotide kinase and RNA ligase, we decided to try to map the restricting locus in *E. coli* CTr5X. In this paper, we report that all of the restrictive ability is due to one locus at ca. 29 min on the *E. coli* K-12 genetic map. Using the tetracycline resistance, Tc^r, gene of a closely linked transposon, we have transduced the locus into both *E. coli* B and K-12. They then become restrictive for *rli*$^-$ and *pseT*$^-$ T4. In the process, we discovered that the locus, or a locus very closely linked to it, also causes the restriction of λ bacteriophage and T4 with cytosine DNA.

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MATERIALS AND METHODS

Bacterial and phage strains. The bacterial and phage strains used and their relevant characteristics are listed in Table 1.

Media. Bacteria were grown either in LB broth (1% tryptone, 1% NaCl, 0.5% yeast extract), or M9 medium (5.5 g of Na_2HPO_4, 3.0 g of KH_2PO_4, 0.5 g of NaCl, 1.0 g of NH_4Cl, 0.2% glucose, 1 mM $MgSO_4$), or M9S medium (M9 medium supplemented with 1% Casamino Acids). LCG was the medium of Ross and Howard-Flanders (19).

Mutagenesis. Mutagenesis was by the method of Adelberg et al. (1).

Conjugation. For Hfr × F$^-$ matings, we mixed 1 ml of log-phase cells of the donor strain (grown without shaking) and 3 ml of the recipient strain in 150-ml Erlenmeyer flasks and incubated the mixture at 37°C for 2 h without shaking. After mating occurred, the culture was vortexed, and the recombinants were selected on appropriate selective plates by counterselecting the donor with streptomycin. Before testing for the *prr* locus, the recombinants were purified by being patched twice on selective plates. They were then cross-streaked against phage at 10^9 cells per ml, and the plates were incubated at 37°C.

Transduction. Transducion by P1 was by the method of Miller (18) with P1*vir*. The cells were washed two or three times with saline to remove unabsorbed viruses before being mixed with top agar and poured on tetracycline (10 μg/ml) LB plates. The Tc^r transductants were patched once on tetracycline plates before being tested for the *prr* locus as above. T4 transduction was as described by Takahashi and Saito (27).

Isolation of Tc^s BJMn10 derivatives. The method of Maloy and Nunn (17) was used for the isolation of tetracycline-sensitive (Tc^s) BJMn10 derivatives.

Preparation of spheroplasts. Spheroplasts were prepared by the method of Weiss (28). The spheroplasts were visualized under a phase scope and counted with a Petroff-Hausser counting chamber. Greater than 99% of the cells had been converted to spheroplasts by the treatment.

Rate of protein synthesis. To label proteins, the cells were grown at 30°C in M9 medium supplemented with tryptophan and methionine (50 μg/ml) and thiamine (10 μg/ml). At 4 ×

TABLE 1. Bacteriophage and bacterial strains

Strain	Genotype	Source or reference
Phage		
T4*pseT2*	Point mutation in *pseT*	10
T4 *rli*-13	Point mutation in gene 63	21
λ*c*I857	*ts* mutation in gene *cI*	Our collection
T4 Dec8	*amE*51 *nd*28(*denA*) rIIH23(*denB*) *alc*8	Our collection
E. coli		
RH041	Hfr *purB67 relA1 thi-1* λ⁻ *spoT1*	B. J. Bachmann
PK191	Hfr *thi-1 relA1*? DE5 λ⁻ *supE44*	B. J. Bachmann
KL983	Hfr *xyl-7 lacY-1 mglP1* λ⁻	B. J. Bachmann
KL96	Hfr *thi-11 relA1* λ⁻ *spoT1*	B. J. Bachmann
B7	Hfr *relA*? λ⁻	P. Kuempel
PLK457	Tn*10*$_2$ in *rac* at 29.7 min	4
B834	*E. coli* B *met*⁻ r_B^- m_B^-	30
K803	*met supE* r_k^- m_k^-	30
B834 *galU56*	*galU* derivative of B834	20
BJMn10	Tc^r restrictive transductant from the cross CTr5X × B834	This study
S655	*gal*⁻ (λ*c*I857*sus*S7)	G. Smith
CTr5X		10

10^8 cells per ml, they were infected at a multiplicity of infection (MOI) of 10 with T4 which had been purified on a CsCl step gradient. Samples of 1 ml of infected cells were labeled with 10 μCi of ^{14}C-labeled amino acid mixture per ml (55 mCi/m atom carbon) at the times and for the periods indicated. Ice was added at the end of the pulse, the cells were spun and resuspended in 0.05 ml of water, and 0.15 ml of Studier cracking buffer (26) was added to each sample before it was boiled for ca. 2 to 3 min to clear.

Gel electrophoresis. Gel electrophoresis was done by the method of Laemmli (16), adapted for slab gels. The clear cell lysates (15 to 20 cells per μl) were loaded on a 9% sodium dodecyl sulfate-polyacrylamide gel and electrophoresed for 3 to 4 h with constant cooling. The gel was fixed by being soaked in 12% trichloroacetic acid for 0.5 h and stained with Coomassie blue (0.05% Coomassie blue, 9% acetic acid, 4% methanol) overnight. The gel was then destained in 7% acetic acid, dried, and autoradiographed.

Radioactive labeling and purification of bacteriophage. To make ^{32}P-bacteriophage λ, the lysogen *E. coli* S655 was grown at 30°C in tryptone broth to 4.0×10^8 cells per ml. The culture was induced for 10 min at 42°C and brought to 37°C. Carrier-free ^{32}P (1 mCi) was added to 100 ml of induced culture, and the cells were shaken at the same temperature for 3 to 4 h. The cells were concentrated 50 times, resuspended in LCG medium, lysed with lysozyme and chloroform, and treated with RNase and DNase. After being cleared by centrifugation, the treated lysate was purified on a CsCl step gradient. To label T4 with cytosine DNA, 100 ml of *E. coli* B834 *galU56* was grown at 37°C to log phase (4×10^8 cells per ml) in M9S, and the culture was infected with T4 Dec8 at an MOI of 2 to 5. Five minutes postinfection, [^{3}H]thymidine (100 μCi; 47 Ci/mmol) was added, and the culture was shaken for 4 to 5 h before chloroform was added. The T4 were concentrated by being centrifuged for 1 h at $39,000 \times g$ and resuspended in 2 ml of M9 buffer overnight. After being cleared by a brief centrifugation, the phage were purified on a CsCl step gradient.

Zone sedimentation in sucrose gradients. To analyze λ DNA after infection, the cells were grown in LCG medium at 37°C to 4×10^8 cells per ml. They were infected with ^{32}P-labeled bacteriophage λ at an MOI of 3. After 10 min at 37°C, the infected cells were centrifuged and washed 3 times with LCG medium. They were then lysed and prepared for sucrose gradient centrifugation as described by Ross and Howard-Flanders (19). Sedimentation was for 16 h at 15,000 rpm in an SW27 rotor. To analyze T4 DNA after infection, the cells were grown in M9S at 37°C to 4×10^8 cells per ml and infected at an MOI of 5. After 10 min at 37°C, the infected cells were centrifuged and washed 3 times with M9S to remove unabsorbed phage. They were lysed and processed by the method of Depew and Cozzarelli (10). The neutral sucrose gradients were 5 to 20% sucrose in a volume of 32 ml with a 2-ml cushion of 70% sucrose and contained, in addition to sucrose, 10 mM Tris-chloride (pH 7.4), 10 mM NaCl, and 10 mM EDTA (pH 7.0). The alkaline sucrose gradients were done by the method of Depew and Cozzarelli (10). Marker phage DNAs were released from the phage by being heated to 60°C in 1% sodium dodecyl sulfate before being layered on gradients.

TABLE 2. Mapping of the *prr* locus by conjugation[a]

Donor	Recombinant scored	No. scored	% Linkage[b]
RH041	His⁺	43	27.9
KL96	His⁺	37	14.2
KL983	His⁺	115	10.4
PK191	His⁺	120	<1.0
B7	Trp⁺	120	<1.0

[a] The recipient in each case was strain CTr5X.
[b] Percentage of the His⁺ or Trp⁺ recombinants which were permissive for T4 *pseT2* and *rli-13*.

Rate of RNA synthesis. To measure the rate of λ RNA synthesis, the cells were grown in LCG medium to 4×10^8 cells per ml at 37°C and infected at an MOI of 3. The infected cells were pulse labeled with [^{3}H]uridine (10 μCi/μg and 1 μg/ml) from 10 to 13 min after infection. To measure the rate of T4 RNA synthesis, the cells were grown in M9S medium, infected at an MOI of 10, and labeled as for λ but from 5 to 8 min after infection. After being labeled, the cultures were poured onto ice. The procedures for RNA extraction and hybridization were as described by Bolle et al. (6). Hybridization was for 4 or 6 h for bacteriophages λ and T4, respectively.

RESULTS

Genetic mapping of the restricting locus in *E. coli* CTr5X. Our strategy for mapping the restricting locus in *E. coli* CTr5X was first to isolate auxotrophic mutations of CTr5X after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and then to cross these with Hfr strains. The prototrophic recombinants were tested by cross streaking to see whether they were restrictive for *pseT*⁻ and *rli*⁻ T4. We initially chose *his* and *trp* as auxotrophic markers, in part because all *his*⁻ and all *trp*⁻ mutations map together at 44 and 27.5 min, respectively (3). It turned out that these were happy choices, since the locus seemed to map between them. About 30% of the His⁺ recombinants with the Hfr strain RH041, which transfers clockwise from 98 min, were no longer restrictive, suggesting that the locus lies somewhere from 98 min clockwise to 43 min (Table 2). The observation that ca. 10% of the His⁺ recombinants with both strains KL96 and KL983, which transfer counterclockwise from 45 and 51 min, respectively, are no longer restrictive suggests that the locus lies close to

his, probably on the *trp* side. A map position in this region is also supported by the results with Hfr PK191 and Hfr B7, which transfer clockwise from 43 min and counterclockwise from 29 min, respectively. Neither Hfr strain transferred the region of the locus early, indicating that the restricting locus lies somewhere between their origins of transfer, i.e., between 29 and 43 min. Because some of the recombinants were no longer restrictive, we tentatively assumed that all of the restrictive ability of CTr5X is due to one locus and named the locus *prr* for polynucleotide kinase-RNA ligase restrictive.

In *E. coli* K-12, the region between 29 and 43 min carries few convenient genetic markers which could be used to further localize the *prr* locus. However, a number of Tn*10* insertion mutants in this region have been isolated (4, 12). We performed transduction experiments with some of these as donors and *E. coli* CTr5X as recipient, selecting for Tc^r. One of the Tn*10* insertions, the $Tn10_2$ of Binding et al. (4) which maps at 29.7 min, gave 1 transductant out of 57 which was no longer restrictive, indicating that the *prr* locus was within 2 min of the site of insertion of the transposon. However, few transductants were obtained with CTr5X as recipient, so it was difficult to accurately determine the cotransduction frequency.

If the $Tn10_2$ insertion lies close to the *prr* locus, it should be possible to use it to transduce the locus into other normal laboratory *E. coli* strains. Accordingly, we used one of the Tc^r transductants of CTr5X which was still restrictive as donor to transduce a number of *E. coli* K-12 strains as well as one B strain, *E. coli* B834. For both the B and K-12 recipients, about 30% or more of the Tc^r transductants were restrictive. However, we noticed one difference between transductants of *E. coli* K-12 and of *E. coli* B. The B strains were only noticeably restrictive at temperatures around 30°C or below, whereas the K-12 strains were, like CTr5X, restrictive at temperatures as high as 37°C. We have no explanation for why the cotransduction frequency into *E. coli* CTr5X (~1%) was lower than out of it (~30%), but it probably reflects differences between CTr5X and other *E. coli* strains in this region.

Because the full restrictive ability of *E. coli* CTr5X can be transduced into other *E. coli* strains selecting for $Tn10_2$, which maps at 29.7 min, we assume that the genetic maps of K-12 and CTr5X are fairly similar in this region and that only one locus in CTr5X, close to 29 min, is making it restrictive. For most of the experiments to be discussed below, we used one of the restrictive transductants of *E. coli* B834, which we shall subsequently refer to as *E. coli* BJMn10.

Presumptive deletions of the *prr* locus. The proximity of a Tn*10* transposon to the *prr* locus in strain BJMn10 raised the possibility of selecting deletions of the locus. A method has been described to select for Tc^s derivatives (see above), many of which are deletions of the Tc^r gene in Tn*10* which extend outward into neighboring chromosomal regions (5). When we selected Tc^s derivatives of *E. coli* BJMn10, we found that more than 50% of them were no longer restrictive (W. Rice and M. A. Jabbar, unpublished data). This suggests that the *prr* locus either represents a gene, or genes, unique to CTr5X and its relatives or is an altered form of a normal nonessential *E. coli* gene, since deletion of the locus presumably causes loss of the phenotypes. However, other explanations are not excluded.

Effect of the *prr* locus on the development of *pseT*$^-$ and *rli*$^-$ T4 mutants. We wished to see whether the defects exhibited by *rli*$^-$ and *pseT*$^-$ T4 in *E. coli* CTr5X, as enumerated above, were the same in strain BJMn10, which had obtained the *prr* locus by transduction. As in CTr5X, both T4 DNA replication and T4 late gene expression were sharply reduced after an *rli*$^-$ mutant infected *E. coli* BJMn10. The results for late gene expression are shown in Fig. 1. Also, as in CTr5X, *stp*$^-$ mutations suppressed the defects in BJMn10, since double-mutant phage with either *rli*$^-$ and *stp*$^-$ mutations or *pseT*$^-$ and *stp*$^-$ mutations plate with 100% efficiency on *E. coli* BJMn10 (data not shown). Thus, the effects of the *prr* locus on *rli*$^-$ and *pseT*$^-$ T4 and the suppression by *stp*$^-$ mutations of these effects seem to be direct and independent of the strain harboring the locus.

Effect of the *prr* locus on the development of bacteriophage λ. Because we were able to transduce the *prr* locus into other *E. coli* strains, we could ask whether any other phenotypes were due to the *prr* locus. We knew, for example, that bacteriophage λ plates with an efficiency of 10^{-2} or less on *E. coli* CTr5X. To ask whether the restriction of λ was due to the *prr* locus, we tested 100 of the Tc^r transductants of *E. coli* B834, of which BJMn10 was a member. We found that those transductants which restricted *pseT*$^-$ and *rli*$^-$ T4 mutants also restricted λ, and vice versa. Furthermore, the Tc^s deletions which no longer restricted *rli*$^-$ and *pseT*$^-$ T4 mutants also no longer restricted λ. We conclude that the restriction of λ is due to the *prr* locus itself or a locus very closely linked to it.

Even though the plating efficiency of λ on strains with the *prr* locus was reduced, the plaques which appeared were almost normal sized. To determine whether they were due to mutants, we plaque purified some of them on *E. coli* B834 and BJMn10. We then replated them on both *E. coli* B834 and BJMn10. If they were plaque purified on BJMn10, they plated with equal efficiency on B834 and BJMn10. However, if they were plaque purified on B834, even after having been plaque purified on BJMn10, they were again restricted on BJMn10 (data not shown). Thus, the plaques on BJMn10 were not due to mutants but to some reversible modification (or lack of it) which permits multiplication in *E. coli* strains with the *prr* locus. Either the DNA is modified or something else injected with the DNA during infection is altered. We

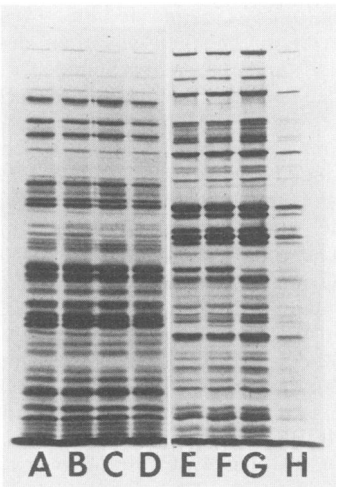

FIG. 1. T4 protein synthesis after infection of *E. coli* B834 and BJMn10 by *rli*$^-$ T4. Autoradiogram of sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Details are described in the text. Labeling was from 8 to 12 min (lanes A through D) and from 38 to 42 min (lanes E through H). Lanes A, C, E, G, *E. coli* B834; B, D, F, H, *E. coli* BJMn10; A, B, E, F, wild-type T4; C, D, G, H, T4 *rli*-13.

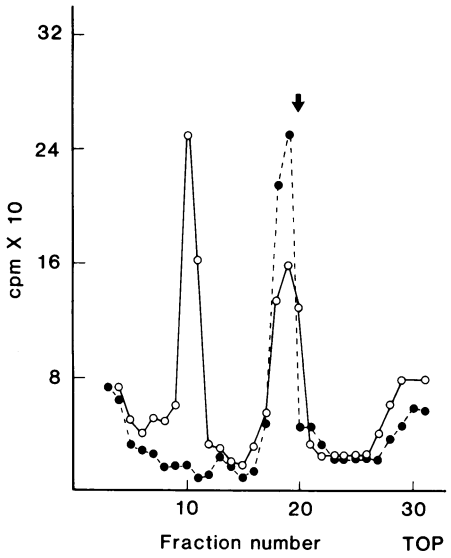

FIG. 2. Neutral sucrose gradient of λ DNA after infection of *E. coli* B834 (○) and BJMn10 (●). Experimental procedures are described in the text. Centrifugation was at 10°C for 16 h at 15,000 rpm in an SW27 rotor. Arrow, Position of mature λ DNA on a parallel gradient.

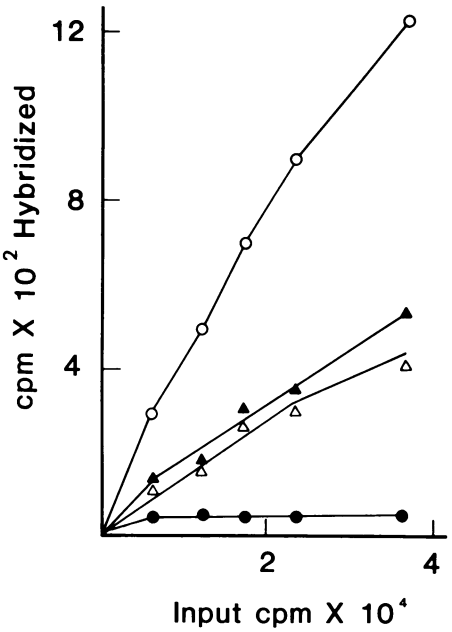

FIG. 3. Hybridization to λ DNA of RNA synthesized after infection of *E. coli* B834 and BJMn10. Experimental details are described in the text. The specific activities of the RNAs were similar, ranging from 13 to 20 cpm/ng. Infection of B834 (○ and △); infection of BJMn10 (● and ▲). Lambda propagated on B834 before infection (○); lambda propagated on BJMn10 before infection (△).

tested other transductants with the *prr* locus and they behaved similarly, indicating that the modification activity, or a regulator thereof, is also due to the *prr* locus or to a locus closely linked to it.

Unlike the block to development of *pseT*⁻ and *rli*⁻ T4 mutants, the block to λ development occurs very early, even before the expression of the λ early genes. The evidence is as follows. When λ infects *E. coli* BJMn10, the λ DNA is ejected from the virus as evidenced by the disappearance of infective λ particles. However, the host cells survive, suggesting that the λ DNA is not being injected into the cytoplasm of the cells or is being quickly degraded after it is injected.

To follow the fate of the λ DNA after infection, we prepared λ with radioactive DNA as described above. We then used the λ to infect *E. coli* B834 and BJMn10. Five minutes later, the cells were chilled, centrifuged, washed, and recentrifuged to remove unadsorbed λ particles. They were then lysed, and the state of the DNA was analyzed by sucrose gradient centrifugation (Fig. 2). After infection of *E. coli* B834, the radioactivity was found in two peaks, in agreement with the results of others (19). The leading peak was due to supercoiled, covalently closed circular DNA, and the trailing peak was due to relaxed circular and linear DNA; the latter forms did not resolve on these gradients. After infection of strain BJMn10, all of the λ DNA was in the trailing peak and so was in either the relaxed circular or linear state. Two points should be made with regard to Fig. 2. One is that the total counts on both gradients are approximately equal, so the λ DNA was apparently injected into the BJMn10 cells and hence not washed off during centrifugation. The other is that the length of the λ DNA after infection of BJMn10 appears to be approximately normal, so there was no substantial degradation of the λ DNA after infection.

To determine whether λ early gene expression was defective in *E. coli* BJMn10 (as suggested by the observation that the host cells survive), we labeled the RNA after infection and hybridized the extracted RNA to λ DNA. (Fig. 3). Substantially less λ RNA was made after infection of strain BJMn10 than after infection of strain B834. It is not clear whether the defect in λ transcription is sufficient to explain the survival of the host cells. We have not analyzed the low amount of λ RNA which is made to see whether it comes from all or only a selected part of the λ early region. The results with the modified λ propagated in BJMn10 are also shown in Fig. 3. The rate of λ transcription after infection of BJMn10 by the modified phage was essentially normal. We are not certain that the reduced rate of transcription after infection of B834 by the modified phage is significant. It may merely reflect differences in the preparation of the viruses.

Effect of the *prr* locus on the development of T4 with cytosine-substituted DNA. Normally, T4 DNA has 100% of its cytosine in the form of hydroxymethylcytosine with a glucose attached to the hydroxymethyl group. In light of the role of a modification in overcoming the restriction of λ, we wondered whether T4 with cytosine DNA would be likewise restricted in *E. coli* with the *prr* locus. Generally, T4 which can multiply with cytosine DNA have an amber mutation in gene 56 which gives the phage the convenient property of having cytosine in their DNA when replicating in a nonsuppressing host and having hydroxymethylcytosine when replicating in an amber-suppressing host. When one such phage, T4 Dec8 (Table 1), was plated on strain BJMn10 without a suppressor, it gave a plating efficiency of only 10^{-5}. On another strain in which the *prr* locus had been transduced into a derivative of *E. coli* B834 with an amber suppressor, the plating efficiency was about 1.0. Thus, T4 Dec8 with cytosine DNA is restricted on *E. coli* with the *prr* locus, but the same phage with hydroxymethylcytosine DNA is not restricted.

The restriction of T4 with cytosine DNA is reminiscent of the restriction of λ. The infective particles disappear, but the host cells survive. Furthermore, there is little or no expres-

sion of the T4 early genes. In Fig. 4, we show an experiment in which proteins were labeled after T4 infection and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. After infection of *E. coli* B834, only T4 early proteins were labeled, as expected, since T4 shuts off host protein synthesis (Fig. 4, lane A). However, after infection of strain BJMn10, T4 early proteins were not labeled and host protein synthesis continued unabated (Fig. 4, lane B). We also labeled RNA after T4 infection and hybridized the extracted RNA to T4 DNA. A much lower percentage of the labeled RNA was complementary to T4 DNA after infection of BJMn10 than after infection of *E. coli* B834 (data not shown).

A simple explanation for the results shown in Fig. 4 is that the T4 DNA is being ejected into the medium or into the periplasmic space of *E. coli* BJMn10, where it cannot be expressed. To follow T4 DNA after infection, we prepared T4 Dec8 phage with radioactive DNA as described above and used them to infect *E. coli* B834 and BJMn10. After 5 min, the cells were chilled, centrifuged, washed, and recentrifuged. The cells were then lysed, and the DNA was analyzed by alkaline sucrose gradient centrifugation (Fig. 5). Again, as with λ, the T4 DNA was not extensively degraded after infection of BJMn10; the single-strand DNA length was almost the same as after infection of *E. coli* B834. Furthermore, the total number of counts per minute on both gradients was almost the same, so the DNA was not washed off during centrifugation. We also performed a variation on the above experiment in which we treated the infected cells with 100 μg of pancreatic DNase I per ml before gradient centrifugation with no loss of acid-precipitable radioactivity, indicating that the T4 DNA is not outside the cells.

The formal possibility remains that the T4 DNA was injected into the periplasmic space. The high molecular weight of the T4 DNA argues against this, since in other situations where T4 DNA is trapped in the periplasmic space, it is extensively degraded by the DNase I of *E. coli* (2). However, to eliminate this possibility, we prepared spheroplasts of infected cells as described above and treated them with 100 μg of pancreatic DNase per ml for 20 min at 37°C, with no loss of acid-precipitable radioactivity. We were left with the same paradoxical situation as with λ; the T4 DNA seems to be in the cytoplasm of the cells, is high molecular weight, but is being ignored by the transcription apparatus.

Further evidence that T4 can inject DNA into strain BJMn10 came from transduction experiments. Phage such as T4 Dec8 can be used to transduce plasmids from one cell to another (27, 29). In Table 3, we show the results of using T4 Dec8 to transduce the plasmid pBR322 from *E. coli* B834 into *E. coli* B834 and BJMn10. Transductants were obtained with the same frequency with both recipients. Therefore, the plasmids, which contain cytosine, can be expressed and replicate after injection into BJMn10.

Anticipating that the restriction of T4 might be subject to the same modification as the restriction of λ, we investigated the T4 plaques which appear at a low frequency on BJMn10. In contrast to λ, these were due to true T4 mutants which could now multiply in BJMn10. However, a modification of the DNA is involved. When the DNA was extracted from T4 Dec8 and from mutants of T4 Dec8 which could multiply in *E. coli* BJMn10 and then was subjected to degradation by a number of different restriction endonucleases, including

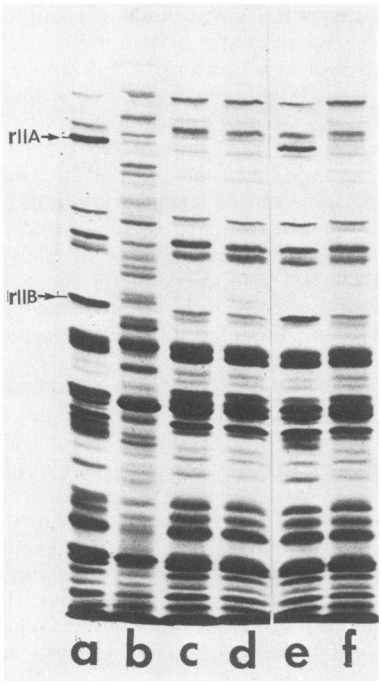

FIG. 4. Electropherogram of proteins synthesized after cytosine-containing T4 infection of *E. coli* B834 and BJMn10. Proteins were labeled as in Fig. 1 but from 5 to 8 min. Lanes A, C, E, Infection of *E. coli* B834; B, D, F, infection of BJMn10. Lanes A and B, Infection by a derivative of T4 Dec8 which has the SAΔ9 deletion (*denB*) instead of rIIH23 (*denB*) and so can synthesize rIIA and rIIB. Lanes C and D, Infection by a mutant of T4 Dec8 which can multiply on BJMn10. Lanes E and F, Mixed infection with the phage strain which can multiply and the strain which cannot multiply in BJMn10. The lines identify the products of T4 genes rIIA and rIIb.

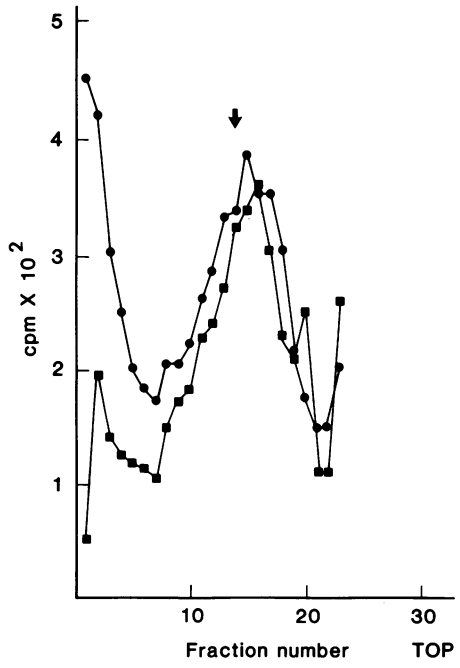

FIG. 5. Alkaline sucrose gradient of cytosine T4 DNA after infection of *E. coli* B834 and BJMn10. Details are described in the text. Arrow, Position of a mature T4 DNA marker on a parallel gradient. *E. coli* B834 (○); *E. coli* BJMn10: (□).

TABLE 3. Transduction of plasmid pBR322 into *E. coli* B834 and BJMn10 by T4 Dec8

MOI	Transduction frequency[a]	
	Strain B834	Strain BJMn10
2.50	3.35×10^{-4}	7.5×10^{-4}
0.25	1.06×10^{-3}	8.1×10^{-4}
0.025	11.0×10^{-3}	1.5×10^{-3}
0.0025	1.25×10^{-3}	5.0×10^{-3}
0.00025	2.0×10^{-3}	5.0×10^{-4}

[a] The number of Ap^r Tc^r transductants per virus. Experimental details are described in the text.

*Hin*dIII, *Pst*I, and *Eco*RI, the mutant DNA was found to be relatively resistant (data not shown). It was, however, sensitive to *Taq*I which, unlike the others tested, will degrade hydroxymethylcytosine-containing T4 DNA (B. Alberts, personal communication).

We also asked whether the modification which permits T4 development can act in *trans* or only in *cis* (Fig. 4). We mixedly infected with a T4 strain which could not multiply by itself in strain BJMn10 and was rII^+ and one which could multiply but had a deletion of the rII genes. If the modification could act in *trans*, then rII gene products would be synthesized; if it could act only in *cis*, they would not be synthesized. It is apparent that the rII polypeptides were synthesized after the mixed infection of *E. coli* B834 (Fig. 4, lane E) but not after infection of BJMn10 (Fig. 4, lane F). Therefore, the modification acts only in *cis*, and unmodified T4 are excluded from gene expression even in mixed infections.

Since their DNA is partially resistant to all but restriction nuclease *Taq*I, it seems likely that the mutants have somehow increased the amount of hydroxymethylcytosine in their DNA and that this is the modification which permits their multiplication in BJMn10. This would not be surprising, since we already knew that T4 Dec8 with hydroxymethylcytosine could multiply in BJMn10. However, the following observation suggests a more intimate involvement of the modification with the restriction in BJMn10. The observation is that it is the type of *unf/alc* mutation which determines whether or not the DNA will be modified.

To multiply with cytosine DNA, T4 must have at least four mutations, three to permit T4 DNA to replicate with cytosine and the fourth, an *unf/alc* mutation, to allow late gene expression from cytosine DNA (25). When T4 with the first three mutations are plated on *E. coli* B834, plaques arise at a low frequency due to spontaneous *unf/alc* mutations. We found that 1 to 10% of these multiplied on strain BJMn10. Considering that phage which can multiply in BJMn10 arise at a frequency of only ca. 0.001% when T4 Dec8 is plated, we conclude that the mutation which permits multiplication in BJMn10 arises at the same time as the *unf/alc* mutation and so is presumably the result of a certain type of mutation which causes the Alc phenotype. The *unf/alc* gene maps between the gene for polynucleotide kinase and RNA ligase (21). Thus, the product of the *unf/alc* gene or another gene between the closely linked *pseT* and *rli* genes would seem to be either doing the modifying or regulating the modifying activity.

DISCUSSION

The bacteriophage T4 RNA ligase (*rli*) and polynucleotide kinase (*pseT*) are nonessential for T4 development in most laboratory *E. coli* strains. However, in a clinical strain, CT196, and a derivative, CTr5X, both enzymes are essential. We mapped the CTr5X locus which causes the restriction of $pseT^-$ and rli^- mutants. A priori this may have proven impossible if the genetic maps of *E. coli* K-12 and CTr5X were too dissimilar or if more than one locus were required. However, although there were minor inconsistencies, almost all of the restrictive ability of CTr5X lies at what would be about 29 min on the *E. coli* K-12 map. We named the restricting locus the *prr* locus.

We used the Tc^r marker of a closely linked Tn*10* insertion mutation to transduce the *prr* locus into other *E. coli* strains. The strains then became restrictive for both $pseT^-$ and rli^- mutants of T4 and were indistinguishable from CTr5X in many respects.

By transducing the *prr* locus into other *E. coli* strains, notably a restrictionless B strain, B834, it was possible to show that other phage were restricted by the *prr* locus or a locus very closely linked to it. Neither λ nor T4 with cytosine DNA could replicate in *E. coli* with the *prr* locus. Those λ which escaped the restriction were modified in a way which permitted them to multiply in hosts with the *prr* locus; so in this respect, the *prr* locus behaves like a classical host restriction-modification system. However, the consequences are very different. In fact, the basis for the restriction is unlike anything described for a bacteriophage-host interaction. The DNA is injected, but not expressed even though not degraded, and, in the case of λ at least, the DNA is not supercoiled. It is as though a very early step in the activation of the DNA is blocked or perhaps the DNA is sequestered, somewhere in the cytoplasm, where it is not accessible to the gyrase and transcription apparatus. Surprisingly, the restriction seems to be specific for the viral DNAs, since plasmid DNA, transduced into a strain with the *prr* locus, seems to be expressed and replicated normally.

In passing, it is worth mentioning another example from procaryotes in which a *cis*-acting modification permits gene expression. The transcription of the *mom* gene of bacteriophage Mu depends upon adenine methylation by the host *dam* methylase (13, 14). However, the restriction and modification of λ by the *prr* locus seemed to be unaffected by dcm^- and dam^- mutations (data not shown).

We do not yet know the nature of the *prr* locus. For example, we do not know whether the same gene product which causes the restriction of rli^- and $pseT^-$ T4 also causes the restriction of λ and T4 with cytosine DNA. All we know is that the genes must be very closely linked as, thus far, they have been inseparable by transduction. We used the closely linked Tn*10* insertion to isolate what are presumably deletions of the *prr* locus. All of the restrictive ability is lost, indicating that an altered nonessential gene of *E. coli* or a gene or genes unique to strain CTr5X, rather than a deficiency in a normal essential *E. coli* gene, is responsible for the phenotypes. This would lay to rest a former hypothesis (22) that the restrictive ability of strain CTr5X is due to an amber mutation in a normal *E. coli* K-12 gene. One possibility is that the *prr* locus constitutes a group of genes, such as a cryptic prophage, unique to CTr5X. The genes for the diverse phenotypes would then appear to be 100% linked because the only homology with *E. coli* K-12 would lie in the flanking sequences. We hope to clone and physically characterize the *prr* locus to distinguish among the many possibilities.

Other T4 mutants are restricted in *E. coli* CTr5X. Among these are T4 with *mot* (D. Hall, personal communication) and plaCTr5X mutations (32). Of these, only *mot* mutants

are restricted on strain BJMn10 (D. Hall, personal communication and our unpublished data). We conclude that the *prr* locus in *E. coli* CTr5X is responsible for the restriction of *mot* mutants but not of plaCTr5X mutants.

Superficially, at least, the polynucleotide kinase and RNA ligase of T4 resemble the RNA-splicing enzymes of eucaryotes (for a discussion see reference 24). The work of David et al. (8, 9) also suggests a role in RNA splicing. They observed the appearance of small, relatively stable, RNA fragments after T4 infection of *E. coli* CT196 and CTr5X (8). There are four fragments, at least two of which can be put together to form a tRNA-like RNA with the cleavage site in the anticodon loop (8). The fragments persist longer after infection by *pseT*$^-$ and *rli*$^-$ mutants, indicating that the kinase and RNA ligase are involved in their further processing (9). The RNA fragments also appear after infection of transductants, such as strain BJMn10, which have received the *prr* locus from CTr5X (M. David, G. Kaufmann, M. Jabbar, and L. Snyder, manuscript in preparation). Thus, the *prr* locus probably either codes for the RNAs themselves or for something required for their appearance after T4 infection, for example, a gene product which interferes with their further processing. An altered RNA ligase would be a good candidate for the product of the *prr* locus. However, the RNA ligase which was discovered recently in uninfected bacteria does not seem to be altered in strains with the *prr* locus (11). Independent of how the *prr* locus causes the appearance of the tRNA-like fragments, the simplest interpretation of the results of David et al. (9) is that the fragments inhibit some reaction and therefore T4 development, if they are not further processed by the T4 RNA ligase and polynucleotide kinase. This, of course, would not easily explain many of the other phenotypes associated with the *prr* locus, including the restriction of λ and T4 with cytosine DNA and the role of DNA modification in overcoming the restriction, since these phenotypes do not depend on T4 gene expression. However, as mentioned above, these other phenotypes could be due to one or more closely linked genes.

Whatever the explanation for the *prr* phenotypes, our work has revealed intriguing similarities between the *prr* region of *E. coli* CTr5X and the *rli*$^-$ *pseT* region of the T4 genome (7). As mentioned, the *pseT* and *rli* genes are closely linked. However, at least one other gene, the *unf/alf* gene, maps between them (21). The *unf/alc* function also blocks the transcription of DNA with cytosine (15, 25). Furthermore, as shown here, the *unf/alc* region may also encode or regulate a DNA-modifying activity which allows T4 to escape the *prr* restriction, and the *prr* region may also encode or regulate a DNA-modifying activity which allows λ to escape the restriction. These analogies may be merely coincidental; but regardless of their relationship, both the T4 and the *E. coli* regions seem to affect reactions which have heretofore been undetected in procaryotes.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant 1 RO1 GM 28001 from the National Institute of General Medical Sciences.

We thank B. Bachmann and P. Kuempel for bacterial strains and advice and M. David and G. Kaufmann for communicating their results before publication.

LITERATURE CITED

1. **Adelberg, E. A., M. Mandel, and G. C. C. Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N′ nitro-N-nitrosoguanidine in *E. coli* K-12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. **Anderson, C. W., and J. Eigner.** 1971. Breakdown and exclusion of superinfecting T-even bacteriophage in *Escherichia coli*. J. Virol. **8**:869–886.
3. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
4. **Binding, R., G. Romansky, R. Bitner, and P. Kuempel.** 1981. Isolation and properties of Tn10 insertions in the *rac* locus of *E. coli*. Mol. Gen. Genet. **183**:333–340.
5. **Bochner, B. R., H. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss for tetracycline resistance. J. Bacteriol. **143**:926–933.
6. **Bolle, H., R. H. Epstein, W. Salser, and E. P. Geiduschek.** 1968. Transcription during bacteriophage T4 development: synthesis and relative stability of early and late RNA. J. Mol. Biol. **31**:325–348.
7. **Cooley, W., K. Sirotkin, R. Green, and L. Snyder.** 1979. A new gene of *E. coli* K-12 whose product participates in T4 bacteriophage late gene expression: interaction of *lit* with the T4-induced polynucleotide 5′ kinase 3′ phosphatase. J. Bacteriol. **140**:83–91.
8. **David, M., G. D. Borasio, and G. Kaufmann.** 1982. Bacteriophage T4-induced anticodon-loop nuclease detected in a host strain restrictive to RNA ligase mutants. Proc. Natl. Acad. Sci. U.S.A. **79**:7097–7101.
9. **David, M., G. D. Borasio, and G. Kaufmann.** 1982. T4 bacteriophage-coded polynucleotide kinase and RNA ligase are involved in host tRNA alteration and repair. Virology **123**:480–483.
10. **Depew, R. E., and N. R. Cozzarelli.** 1974. Genetics and physiology of bacteriophage T4 3′-phosphatase: evidence for involvement of the enzyme in T4 DNA metabolism. J. Virol. **13**:888–897.
11. **Greer, C. L., B. Javor, and J. Abelson.** 1983. RNA ligase in bacteria: formation of a 2′ 5′ linkage by an *E. coli* extract. Cell **33**:899–906.
12. **Harayama, S., E. T. Palva, and G. L. Hazelbauer.** 1979. Transposon-insertion mutants of *E. coli* K-12 defective in a component common to galactose and ribose chemotaxis. Mol. Gen. Genet. **171**:193–203.
13. **Hattman, S., M. Goradia, C. Monaghan, and A. I. Bukhari.** 1983. Regulation of the DNA-modification function of bacteriophage Mu. Cold Spring Harbor Symp. Quant. Biol. **37**:647–653.
14. **Kahmann, R.** 1983. Methylation regulates the expression of a DNA-modification function encoded by bacteriophage Mu. Cold Spring Harbor Symp. Quant. Biol. **37**:639–646.
15. **Kutter, E. M., D. Bradley, R. Schenck, B. S. Guttman, and R. Laiken.** 1981. Bacteriophage T4 *Alc* gene product: general inhibitor of transcription from cytosine-containing DNA. J. Virol. **40**:822–829.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145**:1110–1112.
18. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
19. **Ross, P., and P. Howard-Flanders.** 1977. Initiation of recA$^+$-dependent recombination in *E. coli* (λ). I. Undamaged covalent circular lambda DNA molecules in UvrA$^+$ recA$^+$ lysogenic host cells are cut following superinfection with psoralen damaged lambda phages. J. Mol. Biol. **117**:137–158.
20. **Runnels, J., and L. Snyder.** 1978. Isolation of a bacterial host selective for bacteriophage T4 containing cytosine in its DNA. J. Virol. **27**:815–818.
21. **Runnels, J. M., D. Soltis, T. Hey, and L. Snyder.** 1982. Genetic and physiological studies of the role of the RNA ligase of bacteriophage T4. J. Mol. Biol. **154**:273–286.
22. **Sirotkin, K., W. Cooley, J. Runnels, and L. R. Snyder.** 1978. A role in true-late gene expression for the T4 bacteriophage 5′ polynucleotide kinase 3′ phosphatase. J. Mol. Biol. **123**:221–233.
23. **Snopek, T. J., W. B. Wood, M. P. Conley, P. Chen, and N. R. Cozzarelli.** 1977. Bacteriophage T4 RNA ligase is gene 63 product, the protein that promotes tail fiber attachment to the

baseplate. Proc. Natl. Acad. Sci. U.S.A. **54**:158–165.
24. **Snyder, L.** 1983. T4 polynucleotide kinase and RNA ligase. p. 351–335. *In* C. K. Mathews, E. M. Kutter, G. Mosig, P. B. Berget (ed.), Bacteriophage T4. American Society for Microbiology, Washington, D.C.
25. **Snyder, L., L. Gold, and E. Kutter.** 1976. A gene of bacteriophage T4 whose product prevents true late transcription on cytosine-containing T4 DNA. Proc. Natl. Acad. Sci. U.S.A. **73**:3098–3102.
26. **Studier, F. W.** 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. **79**:237–248.
27. **Takahashi, H., and H. Saito.** 1982. Mechanism of pBR322 transduction mediated by cytosine-substituting T4 bacteriophage. Mol. Gen. Genet. **186**:497–500.
28. **Weiss, R. L.** 1976. Protoplast formation in *Escherichia coli*. J. Bacteriol. **128**:668–670.
29. **Wilson, G. G., K. K. Y. Young, G. J. Edlin, and W. Konigsberg.** 1979. High frequency generalized transduction by bacteriophage T4. Nature (London) **280**:80–82.
30. **Wood, W. B.** 1966. Host specificity of DNA produced by *E. coli*: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. **16**:118–133.
31. **Wood, W. B., M. P. Conley, H. L. Lyle, and R. C. Dickson.** 1978. Attachment of tail fibers in bacteriophage T4 assembly. Purification, properties and site of action of the accessory protein coded by gene 63. J. Biol. Chem. **253**:2437–2445.
32. **Wood, W. B., and H. R. Revel.** 1976. The genome of bacteriophage T4. Bacteriol. Rev. **40**:847–868.