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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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 not injected, but phage genes are not expressed and the host cells survive. These phenotypes are unlike anything yet described for a phage-host interaction.

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 Na2HPO4, 3.0 g of KH2PO4, 0.5 g of NaCl, 1.0 g of NH4Cl, 0.2% glucose, 1 mM MgSO4), 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° cells per ml, and the plates were incubated at 37°C.

**Transduction.** Transduction by P1 was by the method of Miller (18) with Plvir. 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° transductants were picked once on tetracycline plates before being tested for the *prr* locus as above. Transduction was as described by Takahashi and Saito (27).

**Isolation of Tc° BJM10 derivatives.** The method of Maloy and Nunn (17) was used for the isolation of tetracycline-sensitive (Tc°) BJM10 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-Hauser 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 ×
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 ^14C-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 ^32P-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 ^32P (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, ^3H[1]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 × 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 ^32P-labeled bacteriophage λ at an MOI of 3. After 10 min at 37°C, the infected cells were centrifuged and washed 3 times with LCM 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.

**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 ^3H[j]uridine (10 μCi/μl 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 3 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-13*. 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

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**TABLE 1. Bacteriophage and bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
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<tbody>
<tr>
<td>Phage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 pseT2</td>
<td>Point mutation in pseT</td>
<td>10</td>
</tr>
<tr>
<td>T4 rli-13</td>
<td>Point mutation in gene 63</td>
<td>21</td>
</tr>
<tr>
<td>λc857</td>
<td>ts mutation in gene cl</td>
<td>Our collection</td>
</tr>
<tr>
<td>T4 Dec8</td>
<td>ame51 nd28 [denA]</td>
<td>Our collection</td>
</tr>
<tr>
<td></td>
<td>rIIH23 [denB]</td>
<td>alc8</td>
</tr>
</tbody>
</table>

**E. coli**

RH041  Hfr purB67 relA1 thi-1 λ- spoT1  B. J. Bachmann
PK191  Hfr thi-1 relA? DE5 λ - supE44  B. J. Bachmann
KL983  Hfr xyl-7 lacY-I mglP1 λ-        B. J. Bachmann
KL96   Hfr thi-11 relA λ- spoT1          B. J. Bachmann
B7     Hfr relA? λ                        P. Kuempel
PLK457 Tnl0, in rac at 29.7 min         4
B834   E. coli B met- rga- m5                30
K803   met supE rga- m5                    30
B834   galU derivative of B834            20
galU56 BJm10 Te- restrictive transductant from the cross Ctr5X × B834 This study
S655   galU (λc857susS7)                   G. Smith
Ctr5X                                       10

**TABLE 2. Mapping of the prr locus by conjugation**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recombinant scored</th>
<th>No. scored</th>
<th>% Linkage</th>
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<tr>
<td>RH041</td>
<td>His*</td>
<td>43</td>
<td>27.9</td>
</tr>
<tr>
<td>KL96</td>
<td>His*</td>
<td>37</td>
<td>14.2</td>
</tr>
<tr>
<td>KL983</td>
<td>His*</td>
<td>115</td>
<td>10.4</td>
</tr>
<tr>
<td>PK191</td>
<td>His*</td>
<td>120</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>B7</td>
<td>Trp*</td>
<td>120</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

*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.
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 Tn10 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 Te\(^{-}\). One of the Tn10 insertions, the Tn10* 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* 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 Te\(^{-}\) 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 Te\(^{-}\) 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*, 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 BJM10.

Presumptive deletions of the prr locus. The proximity of a Tn10 transposon to the prr locus in strain BJM10 raised the possibility of selecting deletions of the locus. A method has been described to select for Te\(^{-}\) derivatives (see above), many of which are deletions of the Te\(^{-}\) gene in Tn10 which extend outward into neighboring chromosomal regions (5). When we selected Te\(^{-}\) derivatives of E. coli BJM10, 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 pse\(^{-}\) and rli\(^{-}\) T4 mutants. We wished to see whether the defects exhibited by rli\(^{-}\) and pse\(^{-}\) T4 in E. coli CTr5X, as enumerated above, were the same in strain BJM10, 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 BJM10. The results for late gene expression are shown in Fig. 1. Also, as in CTr5X, stp\(^{-}\) mutations suppressed the defects in BJM10, since double-mutant phage with either rli\(^{-}\) and stp\(^{-}\) mutations or pse\(^{-}\) and stp\(^{-}\) mutations plate with 100% efficiency on E. coli BJM10 (data not shown). Thus, the effects of the prr locus on rli\(^{-}\) and pse\(^{-}\) T4 and the suppression by stp\(^{-}\) mutations of these effects seem to be direct and independent of the strain harboring the locus.

![FIG. 1. T4 protein synthesis after infection of E. coli B834 and BJM10 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 BJM10; A, B, E. F. wild-type T4; C, D, G, H, T4 rli-13.](http://jvi.asm.org/Downloadedfrom http://jvi.asm.org/Downloadedfrom http://jvi.asm.org/Downloadedfrom)
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 10-3. 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

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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

<table>
<thead>
<tr>
<th>MOI</th>
<th>Transduction frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain B834</td>
</tr>
<tr>
<td>2.50</td>
<td>3.35 x 10^{-4}</td>
</tr>
<tr>
<td>0.25</td>
<td>1.06 x 10^{-3}</td>
</tr>
<tr>
<td>0.025</td>
<td>1.10 x 10^{-3}</td>
</tr>
<tr>
<td>0.0025</td>
<td>1.25 x 10^{-3}</td>
</tr>
<tr>
<td>0.00025</td>
<td>2.0 x 10^{-3}</td>
</tr>
</tbody>
</table>

* The number of Ap' Tc' transductants per virus. Experimental details are described in the text.

HindIII, PstI, and EcoRI, the mutant DNA was found to be relatively resistant (data not shown). It was, however, sensitive to TaqI 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 unmethylated T4 are excluded from gene expression even in mixed infections.

Since their DNA is partially resistant to all but restriction nuclease TaqI, 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 ungT 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 ungT mutation, to allow 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 ungT 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 ungT mutation and so is presumably the result of a certain type of mutation which causes the AIC phenotype. The ungT gene maps between the gene for polynucleotide kinase and RNA ligase (21). Thus, the product of the ungT gene or another gene between the closely linked pseT and rII genes would seem to be either doing the modifying or regulating the modifying activity.

DISCUSSION

The bacteriophage T4 RNA ligase (rII) 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 rII 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 ppr locus.

We used the Tc' marker of a closely linked Tn10 insertion mutation to transduce the ppr locus into other E. coli strains. The strains then became restrictive for both pseT and rII mutants of T4 and were indistinguishable from CTR5X in many respects.

By transducing the ppr locus into other E. coli strains, notably a restrictionless strain B834, it was possible to show that other phage were restricted by the ppr locus or a locus very closely linked to it. Neither nor T4 with cytosine DNA could replicate in E. coli with the ppr locus. Those which escaped the restriction were modified in a way which permitted them to multiply in hosts with the ppr locus; so in this respect, the ppr 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 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 ppr 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 ppr locus seemed to be unaffected by dam+ and dam− mutations (data not shown).

We do not yet know the nature of the ppr locus. For example, we do not know whether the same gene product which causes the restriction of rII 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 Tn10 insertion to isolate what are presumably deletions of the ppr 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 ppr locus constitutes a group of genes, such as an amber mutation in a normal E. coli K-12 gene. One possibility is that the ppr locus constitutes a group of genes, such as the Tn10 insertion, which is unique to strain 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 ppr 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 pLCTr5X mutations (32). Of these, only mot mutants
are restricted on strain BJMn10 (D. Hall, personal communication and our unpublished data). We conclude that the *prs* 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* CTR196 and CTR5X (8). There are four fragments, at least two of which can be put together to form a RNA-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 BJM10, which have received the *prs* locus from CTR5X (M. David, G. Kaufmann, M. Jabbar, and L. Snyder, manuscript in preparation). Thus, the *prs* 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 *prs* locus. However, the RNA ligase which was discovered recently in uninfected bacteria does not seem to be altered in strains with the *prs* locus (11). Independent of how the *prs* locus causes the appearance of the RNA-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 *prs* 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 *prs* phenotypes, our work has revealed intriguing similarities between the *prs* 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 *unfl* gene, maps between them (21). The *unfl* gene also blocks the transcription of DNA with cytosine (15, 25). Furthermore, as shown here, the *unfl* region may also encode or regulate a DNA-modifying activity which allows T4 to escape the *prs* restriction, and the *pr* 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.

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