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A New Gene of *Escherichia coli* K-12 Whose Product Participates in T4 Bacteriophage Late Gene Expression: Interaction of *lit* with the T4-Induced Polynucleotide 5'-Kinase 3'-Phosphatase†

WENDY COOLEY, KARL SIROTKIN,‡ ROBERT GREEN, AND LARRY SNYDER*

Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824

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We isolated five *Escherichia coli* mutants deficient in their ability to support the late (replication-coupled) gene expression of T4 bacteriophage at 30°C. These mutants, which we call Lit mutants, define at least one novel gene at 25 min on the *E. coli* map. They were selected in an attempt to obtain mutants which restrict the growth of T4 mutants deficient in polynucleotide 5'-kinase 3'-phosphatase but not that of wild-type T4 at 37°C. Some of the mutants do have these phenotypes under some conditions. Studies of the block in T4 development in some of the *E. coli* mutants suggest that Lit mutants are affected in a gene product involved in the metabolism of deoxyribonucleic acid nicks or single-strand gaps. None of the Lit mutants is deficient in the major, bacterial, 3'-phosphatase activity in crude extracts.

Some types of bacteriophage transcription may require covalent alterations of the DNA template. For example, T5 induces a DNA 5'-exonuclease which enhances its late transcription (5, 9). Also, late transcription of T4 is greatly enhanced by phage DNA replication (12), and DNA ligase mutations relieve the requirement for DNA replication (13) presumably by preventing the repair of nicks in DNA.

It would be of interest to know whether the types of bacteriophage transcription which require DNA alterations have common features, and whether an analogous type of transcription exists in uninfected bacteria. A first step in addressing this question is to isolate and study host mutants which cannot support a type of bacteriophage transcription that requires DNA alterations. Presumably, some of these host mutants would be deficient in a function which participates in the phage transcription and, hopefully, in an analogous type of host transcription. For the selection, it may be best to start with phage mutant in a known function thought to be involved in transcription, since using the wild-type bacteriophage for the selection is technically difficult and would reveal little about the actual functions involved. The host mutants of interest are those that can propagate wild-type bacteriophage but not the phage mutants.

† Article no. 9027 of the Michigan Agricultural Experiment Station.

‡ Present address: Division of Chemistry, California Institute of Technology, Pasadena, CA 91125.

We have proposed that the T4 polynucleotide 5'-kinase (10, 11) is required, under some conditions, for T4 late transcription (14). This enzyme is also a 3'-phosphatase (3, 14), so it will henceforth be referred to as the T4 polynucleotide 5'-kinase 3'-phosphatase. This enzyme is normally not required for phage development on standard laboratory strains of *Escherichia coli*, since T4 *pseT*$^-$ mutants, which do not induce it (7, 14), multiply almost normally (7, 4, 14). However, T4 *pseT*$^-$ mutants are restricted on *E. coli* CTr5x, a hybrid of *E. coli* K-12 and *E. coli* CT196 (a clinical isolate) (7), because subnormal amounts of late gene products are made (14) and also, possibly, because of a DNA packaging defect (7). Presumably, *E. coli* CTr5x has an amber mutation in a gene whose product is required to support the multiplication of *pseT*$^-$ T4 because, with the acquisition of an efficient amber suppressor, it becomes permissive for all *pseT*$^-$ mutants, even deletions (14). It is tempting to speculate that the host gene with the amber mutation codes for an enzyme which is analogous to the phage polynucleotide 5'-kinase 3'-phosphatase and can normally substitute for it during T4 development. However, other explanations are possible.

Rather than try to map the putative amber mutation in *E. coli* CTr5x which makes it restrictive for *pseT*$^-$ T4 (which could be difficult because the strain is distantly related to *E. coli* K-12), we isolated similar restricting mutants of a convenient laboratory strain of *E. coli* K-12.

These mutants, which we call Lit⁻ mutants, for late inhibitors of T4, are similar in some, but not all, respects to *E. coli* CTr5x. They define at least one, and possibly two, new genes of *E. coli*.

MATERIALS AND METHODS

Bacterial and phage strains. The strains used, their relevant characteristics, and their source or reference are listed in Table 1.

Isolation of Lit mutants. Lit mutants were isolated as mutants which plated wild-type T4 but not a T4 *pseT* deletion mutant, Δ*pseT*1, at 37°C. Potential mutants were subsequently screened to determine whether the restriction originates from the absence of the *pseT* gene or from the absence of another T4 gene included in the deletion.

The mutants we were seeking would not propagate the T4 *pseT* deletion mutant but would be killed by it. The latter presents problems for the selection, which can be dealt with in a number of ways. The first Lit⁻ mutant, *E. coli* MPH5, was isolated using a variation of the "tab" procedure of Takahashi et al. (16). In this procedure, a carefully predetermined number of phage and mutagenized bacteria are plated together, and *E. coli* mutants that survive are tested for the desired phenotype. This method is very sensitive to the amount of phage and bacteria as well as the condition of the plates, and often MPH5 did not survive a reconstruction of the selection conditions. Therefore, we designed a type of sibling selection procedure by which we isolated the remaining four Lit⁻ mutants.

This procedure succeeds because, on an undisturbed plate, the descendants of a mutant bacterium will be clustered and will protect each other by failing to produce the T4 *pseT* deletion mutant progeny. *E. coli* AB2495 were mutagenized with nitrosoguanidine by the procedure of Adelberg et al. (1), and about 10^8 were spread on a tryptone plate with 10^7 T4 Δ*pseT*1. The plate was incubated overnight at 37°C. This plate was then replicated onto another plate on which about 10^9 T4 Δ*pseT*1 had been spread, and this plate was also incubated overnight. About 500 to 1,000 colonies appeared, mostly due to resistant mutants. The MPH5 prototype survived a reconstruction of this procedure even when mixed with 10^8 of the unmutagenized parental cells beforehand. MPH5 mutant colonies grew with a characteristic "lumpy" appearance, like a bunch of grapes, which helped distinguish them from resistant colonies, which tended to be round and often slimy. The lumpy colonies from the mutagenized plates were picked under a dissecting microscope and streaked across dried streaks of both T4 Δ*pseT*1 and T4 wild type, in that order, both at 10^9/ml, and the plates were incubated at 37°C. The mutants that seemed to be cleared by wild-type T4 but not by Δ*pseT*1 were purified by two cycles of "streaking out" and used as indicator bacteria to plate T4 Δ*pseT*1 and wild-type T4 at 37°C. We found about one Lit⁻ mutant for every 200 lumpy colonies tested, and MPH6, -7, -21, and -24 were isolated by this procedure. All five of the Lit⁻ mutants were probably due to independent mutational events because they either originated from different mutagenized stocks or had different phenotypes.

TABLE 1. *Bacteriophage and bacterial strains*

Strain		Relevant characteristics	Source or reference
T4	Δ*pseT*1	Deletion including *pseT*	14
	*pseT*2	Point mutant in *pseT*	7
	BL292	Gene 55 amber	8
	N82	Gene 44 amber	8
	N81	Gene 41 amber	8
	NG576	Gene 52 amber	8
	N134	Gene 33 amber	8
	M69	Gene 63 amber	8
	B17	Gene 23 amber	8
	E727	Gene 49 amber	8
	N54	Gene 31 amber	8
E. coli	CT196	Clinical isolate	7
	CTr5x	Hybrid K-12: CT196	7
	AB2495	F⁻ multiple auxotroph *trp-35 his-4 supE44*	CGSC[a]
	Hfr Broda 8	Transfers clockwise from 8 min	CGSC
	Hfr KL99	Transfers clockwise from 22 min	CGSC
	Hfr KL96	Transfers counterclockwise from 49 min	CGSC
	MA1008	*pyrC46*	CGSC
	W3110	*trp*⁺	
	PCO254	*purB51*	CGSC

[a] CGSC, *E. coli* Genetic Stock Center, Department of Human Genetics, Yale University School of Medicine, New Haven, Conn.

Mapping Lit mutants. The procedure of Curtiss et al. (6) was used for Hfr crosses. P1 transduction was with P1*vir* in tryptone broth supplemented with 2.5 mM $CaCl_2$. The presence or absence of the Lit phenotype among recombinants was determined by streaking across T4 Δ*pseT*1 and T4 wild type as in the isolation procedure.

Assays of *E. coli* 3′-phosphatases. The assay conditions were essentially those of Depew and Cozzarelli (7). The preparation of 3′-[^{32}P]dTMP was from 500 ml of *E. coli* cells labeled for about six generations in Tris medium with 10^{-3} M PO_4 and 15 mCi of $^{32}PO_4$. The cells were centrifuged and incubated overnight in 1% sodium dodecyl sulfate and 0.5 M NaOH at 37°C to degrade the RNA. The extract was neutralized, blended in a Vortex mixer, extracted twice with phenol, and dialyzed against 1 M NaCl and then water. The dialysate was precipitated with 5% (wt/vol) trichloroacetic acid and washed with 80% ethanol and then ether before drying. The pellet was suspended in 2 ml of water and was digested with micrococcal nuclease (Worthington) to 40 μg/ml in 0.01 M Tris (pH 8.7)–2.5 mM $CaCl_2$ for 1 h at 37°C, neutralized, and further digested by adding spleen phosphodiesterase (Worthington) to 50 μg/ml three times, 1 h apart. The 3′-dTMP from the digest was purified by paper electrophoresis in 0.05 M ammonium acetate (pH 3.5), followed by paper chromatography with 1.8% NH_4OH, 62% isobutyric acid, and 10^{-3} M EDTA and by a repeat of the paper electrophoresis (14). Very high backgrounds were observed if the 3′-dTMP was contaminated with either 5′-dTMP or 3′-UMP. Any 3′-UMP

left over from RNA after dialysis will be separated from 3'-dTMP by the above procedure. However, 5'-dTMP is not separable, so the micrococcal nuclease and spleen phosphodiesterase must be free of contaminating DNase activities.

To prepare extracts for the assays, cells were grown to 4×10^8/ml in tryptone broth (10 g of tryptone and 5 g of NaCl per liter of water), and, if infected, phage was added at a multiplicity of infection of 10 for 15 min at 37°C. The cells were chilled, centrifuged, resuspended at one-tenth the volume in 0.01 M Tris (pH 7.5) with 1 mM mercaptoethanol, and lysed by sonication. *E. coli* B extracts (0.1 ml) could be assayed directly, but *E. coli* K-12 extracts first needed to be diluted about 1:100 with resuspension buffer. For further purification, the extracts were cleared by centrifugation for 20 min at 75,000 × *g* and precipitated with an equal volume of 0.5% protamine sulfate. The supernatant fluid was assayed then or purified further by $(NH_4)_2SO_4$ precipitation and DEAE chromatography by the procedure of Becker and Hurwitz (2). Approximately 30 to 40% of the activity in crude extracts from *E. coli* B or K-12 strains was recovered in the DEAE fractions.

Labeling of proteins and DNA. Because of the multiple auxotrophies of the *E. coli* AB2495 parent, Lit$^-$ mutants were difficult to infect synchronously with T4, necessitating the following procedure. First *thy*$^+$ revertants were used. To label proteins, the cells were grown at 37°C to 4×10^8/ml in M9 (5.5 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, 1.0 g of NH_4Cl, 0.5% glucose, 10^{-3} M $MgSO_4$) supplemented with thiamine at 50 μg/ml and all 19 amino acids at 100 μg/ml except methionine. The cells were concentrated 10 times by centrifugation and infected at a multiplicity of 10 with T4 which had been purified on CsCl step gradients to remove ghosts and prevent ghost exclusion. After 4 min for absorption, the infected cells were diluted 1:10 into fresh medium at 30°C and labeled with 10 μCi of [^{35}S]methionine per ml (865 Ci/mmol) at the times and for the periods indicated.

To label DNA, M9 medium supplemented with 1% casein hydrolysate, tryptophan (50 μg/ml), and thiamine (50 μg/ml) was used, and the cells were labeled with [*methyl*-^{3}H]thymidine at 10 μCi/μg (1 μg/ml) to measure the rate of incorporation and 100 μCi/μg (1 μg/ml) to measure the size of DNA. For the measurements of the rate of incorporation, cells were precipitated with 5% trichloroacetic acid, resuspended with 2% KOH, and reprecipitated with 5% trichloroacetic acid before being collected on glass fiber filters.

Alkaline sucrose gradients. The procedures of Depew and Cozzarelli were followed (7), layering 0.1 ml of an unconcentrated lysate on the gradient.

Slab gel electrophoresis and autoradiography. The apparatus and procedure of Studier (15) were used. Gels were stained with Coomassie blue to check the total amount of protein applied to each well.

RESULTS

Isolation of Lit mutants. We isolated five *E. coli* mutants which restrict the growth of the T4 Δ*pseT*1 mutant but not that of wild-type T4 at 37°C. Three of the five mutants (MPH5, -7, and -21) were indistinguishable in some respects. They multiplied with the same generation time as their parent and absorbed T4 normally. Wild-type T4 produced plaques on them with an efficiency of about 0.2 at 37°C, whereas T4 *pseT* deletions such as Δ*pseT*1 plated with an efficiency of less than 10^{-4}. It was perhaps fortunate that we carried out the selection at 37°C because there was no difference between the plating efficiencies of the *pseT* deletions and wild-type T4 at either higher or lower temperatures. At 30°C both wild-type T4 and T4 Δ*pseT*1 were almost totally restricted, plating with efficiencies of less than 10^{-4}. At 42°C, even the T4 *pseT* deletions plated normally. The effect of varying incubation temperature on wild-type T4 plating efficiency of Lit$^-$ mutants was remarkably abrupt, the plating efficiency going from 10^{-8} to 0.2 in the temperature range of 34 to 37°C. None of these three Lit$^-$ mutants was particularly restrictive for the growth of T4 *pseT* point mutants even at 37°C.

The other two *E. coli* mutants (MPH6 and -24) were different in some respects. MPH6 was noticeably restrictive for T4 *pseT* point mutants at 37°C, giving very small plaques at a frequency of about 10^{-2}. It absorbed T4 more slowly than the parental strain, made slimy, opaque colonies, especially on minimal media, and did not plate bacteriophage P1. It was cold sensitive for T4 multiplication, as were the other Lit$^-$ mutants.

MPH24 grew poorly, particularly at 42°C. It was more permissive for wild-type T4 at 30°C than the other Lit mutants. It absorbed T4 slowly and did not plate P1. It was not particularly restrictive for the growth of T4 *pseT* point mutants.

Mapping mutations responsible for the Lit phenotypes. MPH5, -7, and -21 behaved similarly to each other in Hfr crosses and P1 transduction, as though they have mutations in the same gene. With Hfr Broda 8 as donor, approximately 80% of the Trp$^+$ recombinants were Lit$^+$, whereas only 34% of the His$^+$ recombinants were Lit$^+$. With Hfr KL208, only 30% of the Trp$^+$ recombinants were Lit$^+$. Thus, the conjugation data with these donors suggested that the Lit mutation lay counterclockwise and within about 10 min of *trp*. This conclusion was also supported by the mapping data with Hfr KL99, which transferred the region of the Lit mutation very early, placing it clockwise of 22 min. P1 transduction was used to further localize the Lit mutation (Table 2). We found no cotransduction with *pyrC* or with *trp*. There was, however, about 70% cotransduction with *purB* and the Lit$^-$ marker in all three Lit mutants, indicating that all three had mutations at about 25

TABLE 2. *Mapping of lit mutations with P1 transduction*

Donor	Recipient	Selected marker	Unselected marker	No. tested	Percent co-transduction
MPH5	MA 1008	*pyrC*$^+$	*lit*	110	0
W3110	MPH5	*trp*$^+$	*lit*$^+$	148	0
MPH5	PC0254	*purB*$^+$	*lit*	63	78
MPH7	PC0254	*purB*$^+$	*lit*	76	66
MPH21	PC0254	*purB*$^+$	*lit*	39	51

min on the *E. coli* map. Since the mutations were closely linked and had similar phenotypes, we think they arose in the same gene, although we have not established this point with complementation tests. We have tentatively assigned the name *lit* to the gene and given the mutations in MPH5, -7, and -21 the names *lit-5*, *lit-7*, and *lit-21*, respectively. The one Lit$^-$ transductant of PC0254 we tested was similar to the original MPH5 mutant in all of its phenotypes. We take this as additional evidence that a mutation in one gene was causing the multiple phenotypes of MPH5, -7, and -21.

In contrast, the two other mutants, MPH6 and MPH24, were probably double mutants, having *lit* mutations and at least one other unlinked mutation in a cistron whose product interacts with the *lit* function. The evidence for this is as follows. When MPH6 and -24 were crossed with Hfr Broda 8 and Trp$^+$ was the selected marker, most of the recombinants made very poor lawns on plates. If, as suggested by its map position, the mutation crossed out of MPH6 and MPH24 was a *lit* mutation, we would expect the recombinants to accumulate *lit* mutations upon culturing. This prediction was fulfilled, at least for the MPH6 mutant. Furthermore, we were able to isolate a *lit* single mutant as a recombinant from the original MPH6 mutant. This mutant was similar to our other Lit$^-$ mutants, and we call its mutation *lit-6*. It was used for some of the experiments discussed below.

Because of their similarities, we assume that both MPH6 and MPH24 carried *lit* mutations as well as second-site mutations in the same other gene. We think that it is the mutation in this other gene that caused MPH6 and -24 to be restrictive for T4 *pseT* point mutants, because the recombinants, when first isolated (at least in the case of MPH24), restricted T4 *pseT* point mutants but were completely permissive for wild-type T4 at any temperature.

Effect of *lit* mutations on T4 development. Since at least some of our *lit* mutations did not affect bacterial growth or T4 absorption, we could study their effect(s) on T4 development at 30°C to determine why T4 does not multiply on them. The rate of T4 DNA synthesis was not significantly affected (Fig. 1A). Also, alkaline sucrose gradient centrifugation revealed little difference in the single-strand length of the T4 DNA that was made (Fig. 2A). The remaining parts of Fig. 1 and 2 will be referred to below.

Slab gel electrophoresis of proteins and autoradiography can be used to analyze T4 gene expression during infection because only T4 proteins will be labeled after infection, since T4 shuts off host protein synthesis. It can be seen that T4 early gene expression is not significantly affected by the *lit* mutation (Fig. 3A and E). However, there is a dramatic effect on T4 late protein synthesis, especially very late in infection (Fig. 3B, C, and D and F, G, and H). It can be seen that T4 late protein synthesis began normally on the mutant host but then decreased

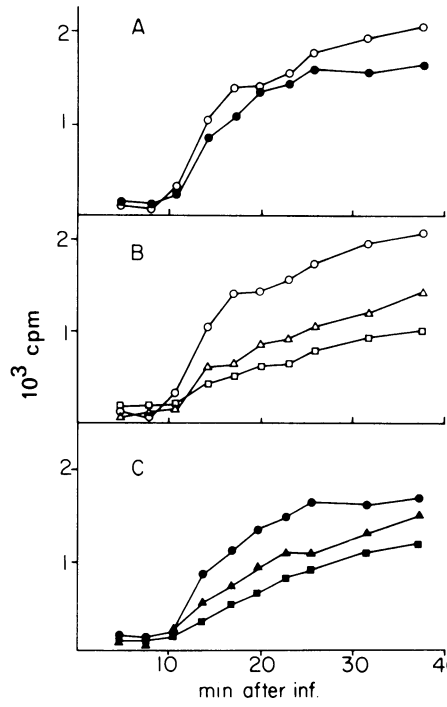

FIG. 1. *The rate of T4 DNA synthesis after infection of the Lit$^+$ parent, E. coli AB2495, and a Lit$^-$ recombinant of MPH6 with T4 wild-type and a T4 Gol mutant. E. coli AB2495, open figures; MPH6 recombinant, closed figures.* (●, ○) *Wild-type T4;* (■, □) *T4 Gol mutant;* (▲, △) *mixed, T4 Gol plus wild-type T4 at a multiplicity of infection of 5 of each. The wild-type T4 experiments shown in (B) and (C) are replots of the data in (A) for comparison. Surviving bacteria were less than 0.1% at 2 min after infection in all cases.*

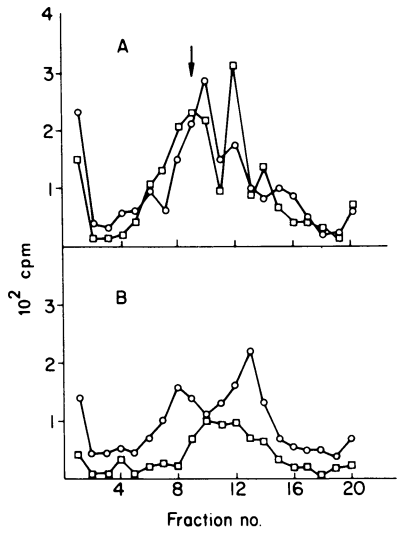

FIG. 2. *Alkaline sucrose gradient centrifugation of T4 DNA pulse-labeled from 14 to 16 min after infection of E. coli by wild-type T4 and a T4 Gol mutant. (A) ○, wild-type T4 on E. coli AB2495; and □, wild-type T4 on the E. coli MPH6 recombinant. (B) ○, T4 Gol on E. coli AB2495; and □, T4 Gol on the E. coli MPH6 recombinant. The arrow shows the approximate position of a viral T4 DNA marker on a similar gradient. Shown are the trichloroacetic acid-precipitable counts.*

in rate relative to the Lit$^+$ control. We assume that the effect on late gene expression is sufficient to explain the inability of T4 to plate on a Lit$^-$ mutant host at 30°C. T4 *pseT* deletion mutants, such as Δ*pseT*1, were no more deficient in late gene expression than wild-type T4 at 30°C (data not shown). We have not yet analyzed protein or DNA synthesis at 37°C.

Physiological and genetic characterization of T4 mutants that multiply on *E. coli* Lit mutants. Plaques formed at a low frequency when wild-type T4 was plated on Lit$^-$ mutants at 30°C or when T4 *pseT* deletions were plated at 30°C or 37°C. These plaques were almost normal size and were due to the growth of T4 mutants because, when the phage from them were isolated, they gave plating efficiencies of 100% on Lit$^-$ mutants. They also gave 100% plating efficiencies on the Lit$^+$ parent. We call the mutants T4 Gol mutants, for grow on Lit. A T4 Gol mutant isolated from any Lit$^-$ mutant plated on any other Lit$^-$ mutant. This was one of the first indications that the *E. coli* Lit$^-$ mutants all had a common molecular basis for their phenotypes.

The frequency of Gol mutants in lysates of some T4 *pseT* deletion mutants was three orders of magnitude higher than in wild-type T4 lysates. There are three possible explanations: the *pseT* deletions may be mutagenic; the Gol phenotype may require two mutations, one of which is in the region included in the *pseT* deletion; or Gol mutants may be selected when the *pseT* deletion mutants multiply, even on a Lit$^+$ host. We think the third explanation, that the Gol mutants were selected, is the correct one because the *pseT* deletion mutants did not accumulate mutations to other phenotypes and because the Gol phenotype did not depend on the *pseT* deletion and could be separated from it by recombination.

In an attempt to map the T4 mutation responsible for the Gol phenotype, we plated the T4 amber mutants shown in Table 1 on *E. coli* MPH5 (*supE*). The phage that made plaques were amber, Gol double mutants; they were crossed with wild-type T4, and the progeny were plated on a *supE*$^+$ but Lit$^-$ derivative of MPH6. Only the Gol, amber$^+$ recombinants should plate, and the recombination frequency between the Gol mutation and the amber mutation can thus be determined. We found no linkage with T4 genes 55, 44, 41, 52, 33, and 63. We had difficulty selecting Gol mutants with amber mutants in genes 23, 49, and 31, so we crossed a Gol mutant with amber mutants in these genes and tested the progeny for wild-type recombinants, since we knew these would be viable. We found very close linkage with gene 23, which is in the late region of the T4 map. Four other independent Gol mutants we tested showed the same linkage to gene 23. We are presently undertaking more precise mapping studies.

Since Gol mutations can almost completely alleviate the affect of the host *lit* mutations on T4 development, studies of T4 Gol mutant development should give us insights into the function of the host *lit* gene product. In particular, if the effect of *lit* mutations on T4 late gene expression is responsible for the inability of T4 to grow on a Lit$^-$ host, then Gol mutations should overcome this effect. Figure 4 shows the results of slab gel electrophoresis and autoradiography of the proteins made late in infection by a T4 Gol mutant. The Gol mutation had no effect on late gene expression in the parental host strain (Fig. 4A and B). However, the Gol mutation did affect late gene expression on the mutant host, completely overcoming the effect of the host *lit* mutation (Fig. 4D and E). It should be noted that some of the T4 late proteins affected (e.g., p37) are coded by genes which are some distance from the site of the Gol mutation and which are separated from it by early genes.

Also shown are the results of a complementation test in which cells were infected by wild-

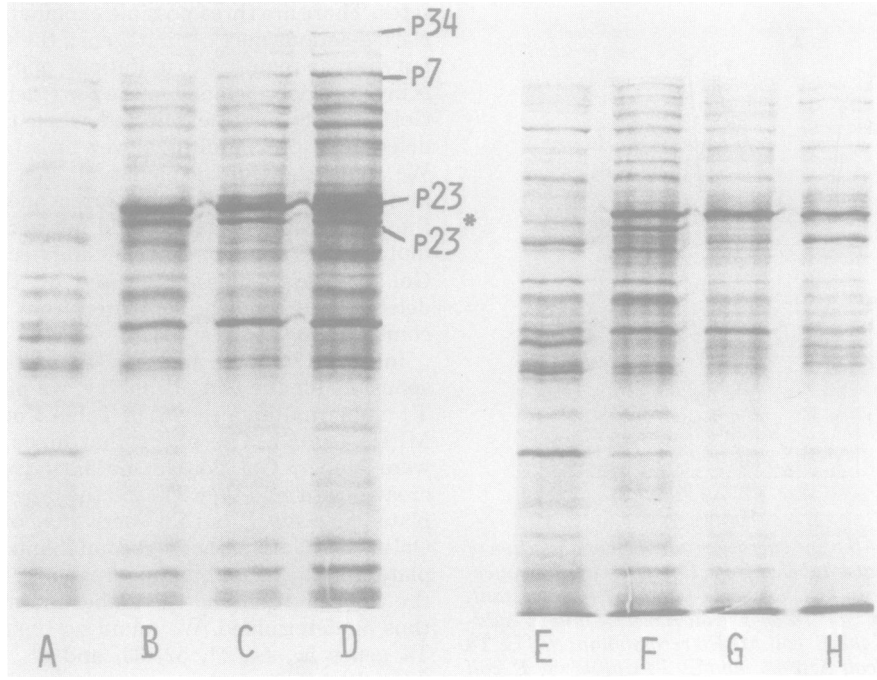

FIG. 3. *Rate of synthesis of T4 proteins after infection of the recombinant of E. coli MPH6. Slab sodium dodecyl sulfate-12% polyacrylamide gels and autoradiograms of proteins pulse-labeled with [^{35}S]methionine after T4 infection at 30°C, multiplicity of infection of 10. Labeling period: (A) and (E), 6 to 9 min; (B) and (F), 18 to 21 min; (C) and (G), 28 to 31 min; (D) and (H), 38 to 41 min. (A to D) E. coli AB2495 Lit$^+$; (E to H) E. coli Lit$^-$. The products of some late genes are identified. P23* is the processed form of the product of T4 gene 23.*

type T4 and the Gol mutant simultaneously and the effect on T4 late gene expression was determined. The Gol mutant was codominant with wild-type T4 for late gene expression on the Lit$^-$ host (Fig. 4F). This is not a special property of this Gol mutation, because two other mutants we have tested were also codominant. It is surprising that codominant mutations could enhance T4 late gene expression since T4 late transcription undoubtedly occurs from many promoters simultaneously and there are many T4 genomes present late in infection. We shall return to possible explanations in the Discussion.

The only T4 Gol mutant we tested also showed a defect in T4 DNA replication, and this occurred on any host. Figure 1B and C show that the rate of T4 DNA synthesis was sharply reduced when a T4 Gol mutant infected the parent *E. coli* AB2495 (Fig. 1B) or a Lit$^-$ mutant (Fig. 1C). Again, the effect of the Gol mutation on the rate of T4 DNA replication seemed to be codominant, because in mixed infections the rate of T4 DNA synthesis was intermediate between the T4 Gol and wild-type T4 rates.

The T4 Gol mutation affected not only the rate of DNA synthesis but also the size of the T4 DNA that was made. In Fig. 2B, alkaline sucrose gradient centrifugation revealed that the single strands of the T4 Gol DNA labeled in a 2-min pulse of [^{3}H]thymidine were significantly shorter in either a Lit$^-$ mutant or its parent. All of the experiments above were done with a T4 Gol mutant which we had shown to have a mutation close to gene 23 in the late region. We have not absolutely ruled out the possibility of two mutations in the Gol mutant, of which one is responsible for the effect on late protein synthesis and the other for the effect on replication. However, this seems very unlikely, since the Gol mutant was a spontaneous mutant and both the effects on late gene expression and replication were codominant.

Effect of *lit* mutations on host 3′-phosphatase activities. We began the isolation of *E. coli* Lit$^-$ mutants with the thought that we might find a host gene that codes for a product analogous to the T4 phage-induced polynucleotide 5′-kinase 3′-phosphatase. If so, Lit$^-$ mutants may be deficient in a similar enzyme. No one has been able to detect a polynucleotide 5′-kinase activity in uninfected bacteria (10, 11). In 1967, Becker and Hurwitz (2) reported a 3′-phosphatase from *E. coli* which was nonspecific for

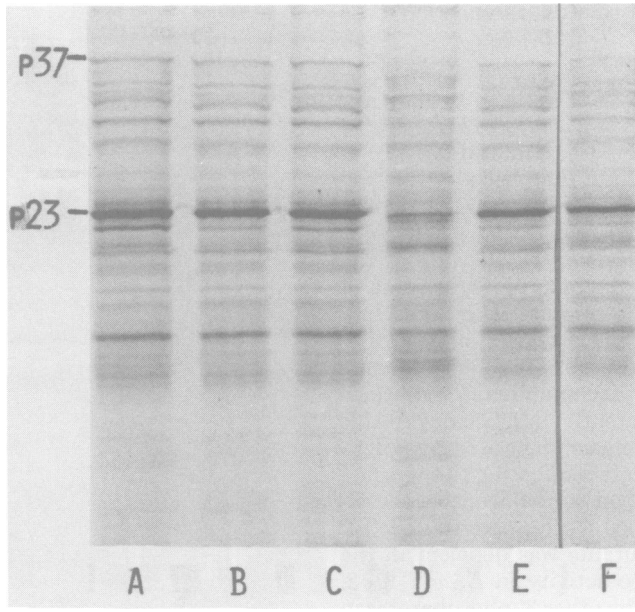

FIG. 4. *T4 late protein synthesis by wild type and a T4 Gol mutant on E. coli AB2495 (Lit⁺) and E. coli MPH7 (Lit⁻). Shown are sodium dodecyl sulfate-polyacrylamide gel electrophoresis autoradiograms as in Fig. 3. Time of labeling, 35 to 38 min after infection. (A) and (D) Wild-type T4, multiplicity of infection of 10; (B) and (E) T4 Gol, multiplicity of 10; (C) and (F) T4 Gol plus wild-type T4, multiplicity of 5 for each. (A to C) E. coli AB2495 (Lit⁺); (D to F) MPH7.*

3′- or 5′-phosphates on mononucleotides, but which, on polynucleotides, would only remove 3′-terminal phosphates. This enzyme was reported to be much more active in extracts of *E. coli* C than *E. coli* B. We found that not only *E. coli* C, but also *E. coli* K-12 and *E. coli* CTr5x, displayed almost 100 times as much 3′-phosphatase activity in crude extracts as *E. coli* B. The major activity in *E. coli* K-12 was not deficient in any of our Lit⁻ mutants or in *E. coli* CTr5x (see Table 3). We think the differences in activities shown in Table 3 are not significant and are due to the difficulty of assaying the enzyme in crude extracts, because they were not reproducible and disappeared upon further purification of the enzyme.

We do not know if the major 3′-phosphatase detected at much lower levels in B strains was due to the same or a different enzyme. It purified much the same through the DEAE chromatography step of Becker and Hurwitz and could use Co^{2+} instead of Mg^{2+} as a divalent cation, as could the K-12 enzyme.

We consistently observed an inhibition of the *E. coli* B enzyme after T4 infection, as indicated by the lower level of 3′-phosphatase activity in T4 *pseT*2-infected cells (i.e., in the absence of phage-induced enzyme) compared to uninfected cells (see Table 3). We think this inhibition is real because it has appeared dozens of times in our assays. The inhibition of the host enzyme was more apparent after partial purification of the enzyme with protamine sulfate (see Materials and Methods), when almost no activity could be detected (data not shown). Thus, the activity which persisted in T4 *pseT*2-infected cells in Table 3 was probably due to other competing activities in crude extracts. In contrast, the major 3′-phosphatase activity in *E. coli* K-12 did

TABLE 3. *3′-Phosphatase activities in Lit mutants and extracts of T4-infected E. coli B*

Expt no.	Source of crude extract	3′-Phosphatase activity[a]
I	*E. coli* AB2495	4.8
	MPH5	5.4
	MPH6	3.3
	MPH7	7.7
	MPH21	5.1
	MPH24	3.2
	CTr5x	8.7
II	*E. coli* B	0.11
	T4⁺-infected *E. coli* B	0.17
	T4 *pseT*2-infected *E. coli* B	0.04

[a] Nanomoles of PO_4 released from 3′-dTMP in 30 min at 37°C per 4×10^8 cell equivalents.

not seem to be inhibited after T4 infection (data not shown).

DISCUSSION

Some Lit⁻ mutants of *E. coli* K-12 restrict T4 *pseT*⁻ point mutants more than wild-type T4 at 37°C. The Lit⁻ mutants that behave this way seem to have mutations in another, as yet uncharacterized, gene. It appears that a deficiency in this other gene was the direct cause of the restriction of *pseT*⁻ mutants of T4 in some of the Lit⁻ mutants. There must be a very close interaction between the products of this other gene and the *lit* gene because Lit⁻ recombinants made poor lawns and accumulated *lit* mutations as suppressors. Apparently, a deficiency in this other function must be compensated by a deficiency in *lit* function. It is tempting to speculate that this other function is analogous to the 5′-polynucleotide kinase 3′-phosphatase of T4. None of the Lit⁻ mutants was deficient in the major 3′-phosphatase activity in *E. coli* K-12. However, there may be other 3′-phosphatases in *E. coli*, as suggested by our observation that an activity in *E. coli* B, which is present at much lower levels, is inhibited after T4 infection, which is not true of the major K-12 activity.

A comparison of Lit⁻ mutants and *E. coli* CTr5x is interesting. In both *E. coli* CTr5x and Lit⁻ mutants, T4 late gene expression is defective. Furthermore, the effect on late gene expression in both is temperature dependent, since *E. coli* CTr5x allows normal synthesis of T4 late proteins after infection by a T4 *pseT*⁻ mutant at 42°C (see Fig. 5). However, T4 *pseT* Gol mutants do not plate on *E. coli* CTr5x. In spite of this, it seems likely that the restriction of T4 *pseT* mutants by *E. coli* CTr5x and by *E. coli* Lit mutants is related.

We think that *lit* mutations define a new cistron of *E. coli* K-12. The only other known mutations that map in this region and restrict T4 are *galU* mutations. However, *galU* mutations cotransduce with *trp* and not *pur*B and do not exert an effect on T4 in the first generation, and the progeny T4 grown on a *galU* host will plate on *rgl* but not *rgl*⁺ *E. coli*. Those T4 progeny that escaped the *lit* restriction plated equally well on *rgl*⁺ and *rgl* cells (data not shown).

The *E. coli lit* mutations prevented late gene expression and the development of bacteriophage T4 at temperatures below 34°C, suggesting that T4 needs the product of the host *lit* gene for its late gene expression, at least at lower temperatures. The plating efficiency of T4 on host *lit* mutants increased abruptly from 34 to 37°C. This abrupt change occurred in all of our

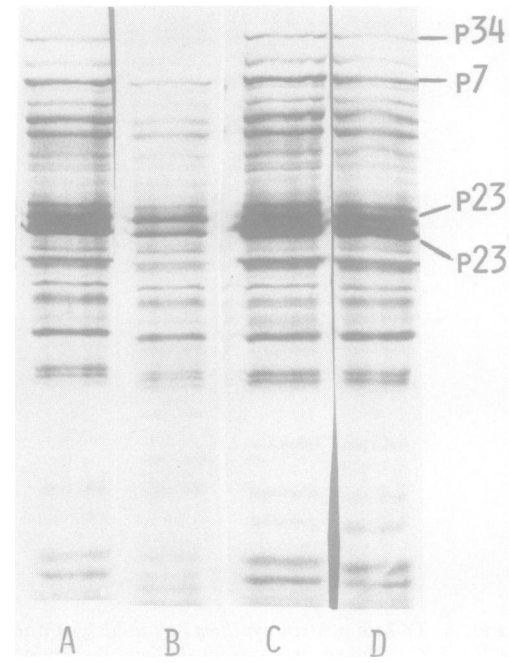

FIG. 5. *T4 late protein synthesis on E. coli CTr5x at 37 and 42°C. Shown are sodium dodecyl sulfate-polyacrylamide gel electrophoresis autoradiograms as in Fig. 3. (A) and (C) Wild-type T4; (B) and (D) T4 pseT2; (A) and (B) 37°C; (C) and (D) 42°C. Time of labeling: (A) and (B), 38 to 41 min; (C) and (D), 28 to 31 min. Multiplicity of infection of 10 throughout.*

lit mutants, even when the *lit* mutation had been transduced into a different genetic background, and is probably a reflection of the function of the product of the *lit* gene.

The participation of the *lit* function in T4 late gene expression presumably occurs at the level of transcription and is the result of altering T4 DNA. Some of the evidence for DNA alteration by the *lit* function comes from the results on the effect of a T4 Gol mutation on T4 DNA replication, since it caused a reduction in the rate of T4 DNA replication and in the length of the newly synthesized DNA even though early gene expression was not affected.

T4 Gol mutations have unusual characteristics. They map in the late region but can affect T4 DNA replication early. They are also codominant with wild-type T4 both in their defect in T4 DNA replication and in their ability to enhance T4 late gene expression on a Lit⁻ host. Explanations for codominance usually invoke either gene dosage effects or *cis*-acting proteins or sites in DNA. The gene dosage explanation for codominance assumes that the Gol function is present in limiting amounts for the phenotype,

so that making one-half as much allows one-half as much late protein synthesis. The gene dosage explanation seems unlikely, but the *cis*-acting site (or protein) explanation is in some ways more radical, since the site of the Gol mutation must influence the expression of all the late genes of T4 even though they are expressed from many promoters, most of which are to the 5′ side of the Gol mutations or separated from them by an early region which is transcribed in the reverse direction. Experiments can be designed which should allow us to distinguish between these two possible explanations for the codominance of T4 Gol mutations.

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