JOURNAL OF VIROLOGY, Dec. 1978, p. 877-884
0022-538X/78/0028-0877$02.00/0

Vol. 28, No. 3
Printed in U.S.A.

Bacteriophage Lambda Mutants (λ*tp*) That Overproduce Repressor

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Received for publication 25 May 1978

Lambda *tp* mutants, selected for their ability to form turbid plaques on *lon* hosts, overproduce repressor. The *tp*1 and *tp*2 mutations have been located within (or adjacent to) the *c*III gene. The *tp*1 mutation reduced late gene expression, as measured by endolysin synthesis (in the absence of functional *c*I repressor) and progeny phage yield. The *tp*4 mutation was mapped in the *c*Y-*c*II region, and complementation tests indicated that *tp*4 affects the diffusible product of the *c*II gene. The *tp*4 mutation also reduced progeny production, but did not markedly affect endolysin synthesis.

The establishment of immunity after λ infection requires expression of the *c*I gene; the products of the *c*II and *c*III genes are essential positive regulators of *c*I expression (5, 24). The promoter from which the *c*I gene is transcribed during immunity establishment (*pre*) might be defined by the *c*Y mutations which act in *cis* to limit *c*I expression. In this model, *c*II and *c*III proteins function to stimulate transcription from the cY^+ region (5, 24). Alternatively, the *c*I transcript could initiate at p_o, the promoter for *oop* RNA synthesis (10). The *c*Y mutations would limit the extension of *oop* RNA to include the *c*I gene.

Use was made of the fact that λ^+ synthesizes less repressor after infection of *lon* hosts than in lon^+ hosts, and consequently forms clear plaques on *lon* indicators, to isolate λ mutants (λ*tp*) which overproduce repressor (36, 38). The λ*tp* mutants were isolated as turbid-plaque formers on *lon* hosts; they form extremely turbid plaques on wild-type hosts (38). Assays of repressor levels demonstrated that the λ*tp* mutants overproduce repressor after infection but that λ*tp* monolysogens contain the same repressor levels as λ^+ monolysogens (36). Thus, the *tp* mutations affect the establishment of repression, but not its maintenance.

The λ*tp* mutations have been mapped by using deletion phages and prophage strains. *tp*1 and *tp*2 map within or very near *c*III, and *tp*4 maps in the *c*Y-*c*II region. By complementation, *tp*4 was found to affect the diffusible *c*II product. The effects of the *tp* mutations on λ late gene expression, as measured by endolysin and phage yield, are also reported.

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MATERIALS AND METHODS

Bacterial and phage strains. *Escherichia coli* K-12 strain AB1157, F^- *thi thr leu arg proA his gal xyl ara mtl lac str* lon^+, was obtained from P. Howard-Flanders, and strain 2e01c, F^- *thi thr leu lac str supE* lon^+, was from M. Malamy. Strain AX14 is a spontaneous *lon* derivative of 2e01c (37). Strain AB2487, *recA*, was obtained from B. Bachmann.

Phage strains and λ lysogens used are listed in Table 1.

Media and buffers. Yeast extract-tryptone (YET) broth, top agar, and bottom agar, supplemented with 1 mM $MgSO_4$, and λ dilution buffer have been described previously (38).

Preparation of λ lysates. λ^+ and λ*tp* mutants were prepared by UV induction of AB1157, 2e01c, or AX14 lysogens. λ*c*I857 was prepared by thermal induction. Lysates of other λ clear-plaque mutants and of λ*bio* phage were made by the soft-agar overlay technique. Soft-agar overlay plates seeded with λ*c*I857*cro*27 and 2e01c or AX14 cells were incubated at 40°C overnight.

Genetic mapping of λ*tp* mutations. In λ*tp* mapping experiments involving lysogens containing deletion prophage strains, the cells were grown to 5×10^8 cells per ml and infected with the phage to be tested at a multiplicity of infection (MOI) of 0.1 to 0.5. After a 20-min adsorption period at room temperature, the infected cells were diluted 1:20 into 0.01 M $MgSO_4$. The suspensions were UV irradiated with an incident dose of 400 ergs/mm^2 and then diluted 1:10 into YET broth. (Infected cultures of strain SA297 were UV irradiated with 100 ergs/mm^2.) The cultures were shaken for 2 h at 37°C and treated with $CHCl_3$, and the phages were plated on AX14 *lon* cells at 37°C. Recombination frequencies are expressed as the percentage of total progeny which had acquired the Tp^+ phenotype, after subtraction of the reversion frequency. The parental prophage did not plate because the prophage strains employed were defective.

In crosses of λ*tp* mutants with nondefective phages, the host strains AB1157 or 2e01c were grown to 3 ×

TABLE 1. *Phages and lysogens*

Strain	Source	Reference
Phages		
λ^+	D. Kaiser	12
λimm^{434}	E. Cox	13
λimm^{21}	E. Cox	17
λcII2002	L. Reichardt	2
λcY42	L. Reichardt	12
λcI857	D. Kaiser	32
λcI857*cro*27	S. Adhya	7
λbio275	W. Szybalski	33
λbio252	W. Szybalski	33
λbio250	W. Szybalski	33
λbio10	W. Szybalski	33
Lysogens		
B912	W. Spiegelman	
SA297	S. Adhya	15
GK85 (M16-3)	D. Kaiser	14
GK90 (R24-2)	D. Kaiser	14
W602 (λM58-2)	F. Blattner	15

10^8 to 5×10^8 cells per ml and infected at a multiplicity of 5 with each phage. After UV irradiation (by the procedure described above), the cells were diluted into YET and incubated until lysis. Progeny from crosses involving λ*tp* and λ*bio* phages were plated on the *recA* strain AB2487 to prevent plaque formation by the *red gam* λ*bio* phages (19, 40); recombination frequency is expressed as the percentage of progeny which acquired the Tp^+ phenotype, after subtracting the frequency of reversion. The recombination frequency between the λ*tp* mutations and the λimm^{21} or λimm^{434} immunity regions was calculated as the percentage of λ*tp* plaques which formed on a $lon(\lambda imm^{\lambda})$ lysogenic lawn.

λ repressor assays. The phage infection procedure, preparation of extracts, and λ repressor-λDNA-nitrocellulose filter binding assay (21, 24) were used as described previously (36).

Measurements of endolysin levels and phage yields. Strain 2e01c *lon*$^+$ cultures were grown at 37°C and infected with λ^+, λ*tp*, λcI857*tp*$^+$, or λcI857*tp* phages at 37 or 40°C for determination of endolysin levels and phage yields as described previously (3, 36). MOI was calculated based on phage titration on strain AB1157(λimm^{434}).

RESULTS

Efficiency of plating of λ*tp* mutants on nonlysogenic and lysogenic hosts. The λ*tp* mutants plated with increased efficiency on heteroimmune lysogens, compared with corresponding nonlysogens, by a factor of 1.4 for λ*tp*1 to a factor of 2 or more for λ*tp*4 (Table 2). The turbid-plaque phenotype of the λ*tp* mutants was not appreciably affected by the indicator used. In addition, λ*tp*4 plated on the nonlysogen strain AB1157 with a higher efficiency than on strain 2e01c. Throughout this study, phage titrations were determined on the indicator AB1157(λimm^{434}).

Genetic mapping of the *tp* mutations. Tp^+ progeny were recovered after λ*tp*1, λ*tp*2, and λ*tp*4 infection of the defective lysogen B912 (Fig. 1; Tables 3 and 4). This strain contains a prophage with a deletion from gene *R* through the right terminus of the prophage map. Therefore, the λ*tp* mutations are not located in genes for λ structural proteins.

λ*tp*1, λ*tp*2, and λ*tp*4 each recombined with λimm^{434} to produce $\lambda tpimm^{434}$ and $\lambda tp^+imm^{\lambda}$ recombinants (Tables 3 and 4) and, thus, lie outside the λ immunity region as defined by the imm^{434} substitution. Therefore, the λ*tp* repressor-overproducing mutants cannot possess alterations in the λ *cro* "antirepressor" gene or its site of action, because these lie within the λ immunity region (6, 7, 22), or in the *c*I repressor gene itself.

The λ*tp*1 and λ*tp*2 mutations were unable to recombine with the defective prophage present in the lysogenic bacterial strain GK85 (Table 3). Strain GK85 carries a deletion from the left end of the prophage map into the immunity region (Fig. 1). Thus, the λ*tp*1 and λ*tp*2 mutations apparently lie to the left of the immunity region. The λ*tp*4 mutation recombined with the GK85 prophage to yield Tp^+ phage (Table 4) and, thus, must lie to the right of the λ immunity region.

Before more detailed genetic mapping was undertaken, a series of crosses of the λ*tp* strains against λimm^{434} and various marked λ strains and backcrosses against λ^+ were made to reduce the likelihood that the λ*tp* mutants possessed, as a result of mutagenesis involved in their isolation, other mutations which might interfere with the analysis of the mutations responsible for the turbid-plaque phenotype on *lon* hosts. λ*tp*1 and λ*tp*4 strains showed no phenotypic variation after repeated backcrossing. λ*tp*2, which forms small turbid plaques on *lon* cells and tiny, barely discernible plaques on *lon*$^+$ cells (38), contained a second mutation which affected plaque size, but not plaque turbidity. Infection of the lysogenic bacterial strain GK85 with λ*tp*2 resulted in no λ*tp*$^+$ recombinants, as mentioned above;

TABLE 2. *Plating efficiency of* λ^+ *and* λ*tp mutants on nonlysogens and on heteroimmune lysogens*

Host strain	Efficiency of plating[a]			
	λ	λ*tp*1	λ*tp*2	λ*tp*4
2e01c	1.0	1.0	1.0	1.0
2e01c(λimm^{434})	1.0	1.4	1.7	2.3
2e01c(λimm^{21})	1.2	1.2	1.1	1.1
AX14*lon*	1.0	1.1	1.1	1.0
AB1157	1.0	1.0	1.0	1.5
AB1157(λimm^{434})	1.2	1.5	1.7	3.0

[a] Plating efficiency on strain 2e01c was used as a standard. Plates were incubated at 37°C.

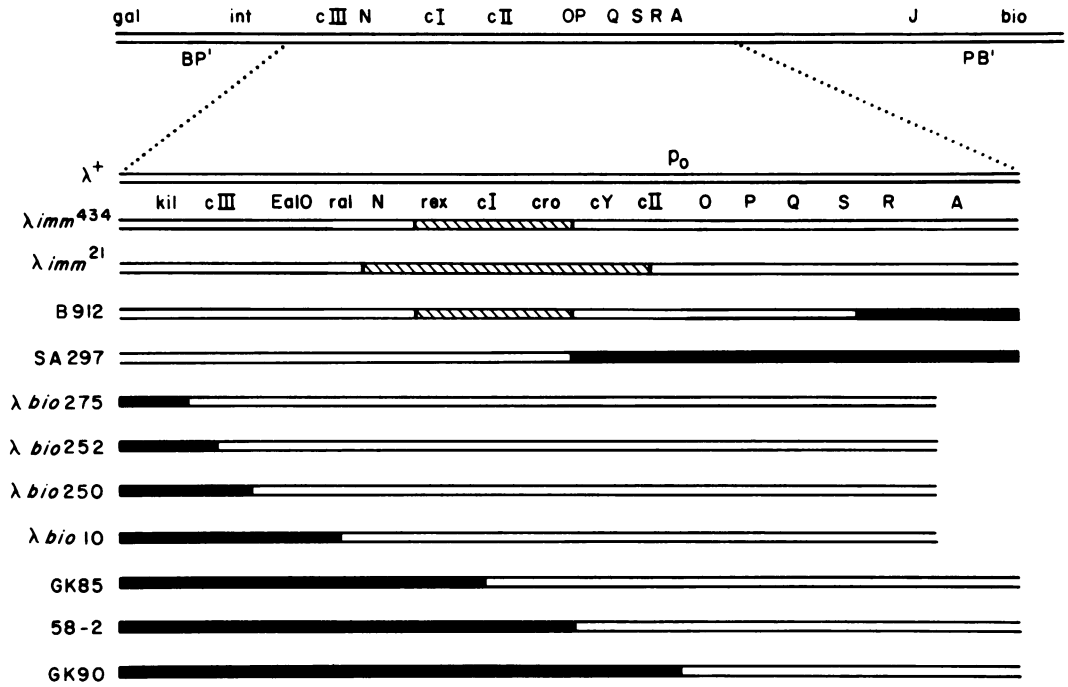

FIG. 1. *Genetic map of λ prophage or phage. White areas represent λ+ sequences, hatched areas are substitutions, and dark areas are deletions. Not drawn to scale.*

TABLE 3. *Mapping of λtp1 and λtp2m*

Phage or prophage	Recombination frequency (%)[a]		
	cIII67	*tp1*	*tp2m*
λ*imm*434	ND[b]	13.6	24.2[c]
λ*imm*21	5.4	5.0	8.1
B912	ND	24.3	45.7
SA297	ND	4.9[c]	4.4
λ*bio*275	1.8	2.0	4.5
λ*bio*252	0.6	0.7	1.8
λ*bio*250	—[d]	—	—
λ*bio*10	—	—	—
GK85	—	—[c]	—

[a] Corrected for reversion as follows: host cells were infected with individual phage strains and UV irradiated, and the progeny were plated to score plaques which were phenotypically λ+. For λ*tp*1 and λ*tp*2*m*, the apparent reversion frequency was 0.05 to 0.1%.

[b] ND means that this cross was not done.

[c] Crosses were made with the original mutant phages which had not been backcrossed.

[d] —, Frequency of recombination was less than 0.1%.

however, a second plaque phenotype was noted in addition to the *tp*2 parental phenotype. These plaques, which occurred at a frequency of 3.6% of the total progeny, were medium-sized, turbid plaques on AX14 *lon* cells and small, easily seen, very turbid plaques on 2e01c *lon*+ cells. A medium-sized, turbid-plaque-producing phage (from a *lon* indicator) was purified from the progeny of this infection and designated λ*tp*2*m*.

λ*tp*2*m* infection of 2e01c *lon*+ cells resulted in repressor levels 80% higher than those after λ+ infection at the same multiplicity (MOI = 3) (data not shown). Thus, λ*tp*2*m* apparently has retained this important characterisitic of the original λ*tp*2 mutant. λ*tp*2*m* was used for further genetic mapping.

The λ*tp*1 and λ*tp*2*m* mutations were mapped by using a series of nondefective λ*bio* phage

TABLE 4. *Mapping of the λtp4 mutation*

Phage or prophage	Recombination frequency (%)[a]			
	*cro*27	*c*Y42	cII2002	*tp*4
λ*imm*434	ND[b]	ND	ND	3.7
λ*imm*21	ND	ND	—[c]	—
B912	ND	ND	ND	37.1
SA297	10	—	—	—[d]
GK85	ND	4.6	2.9	10.0
58-2	—	1.0	1.3	4.0
GK90	ND	ND	—	—[d]

[a] Corrected for reversion frequency as explained in footnote *a* of Table 3. For λ*tp*4, the apparent reversion frequency was 0.05 to 0.1%.

[b] ND means that this cross was not done.

[c] —, Frequency of recombination was less than 0.1%.

[d] These crosses were made with the original λ*tp*4, which had not been backcrossed.

strains which lack varying segments of the λ genome beginning at the left end of the prophage map (33) (Fig. 1). The progeny of mixed infections with λ*tp*1 or λ*tp*2*m* and each of these λ*bio* phages were analyzed after plating on the *recA* strain AB2487. Only λ*tp* parental phage and λ*tp*$^+$ recombinant phage possessing the *red* and *gam* genes will plate on this host.

Both the λ*tp*1 and the λ*tp*2*m* mutations lie between the end points of the λ*bio*252 and λ*bio*250 deletions (Table 3). The *bio*252 deletion ends within *c*III, but λ*bio*252 recombined with λ*c*III67, λ*tp*1, and λ*tp*2*m* to yield wild-type progeny. The *bio*250 deletion removes approximately 25 more nucleotide pairs than *bio*252 (33) and removes also the ability to recombine with *c*III67, *tp*1, and *tp*2*m*. The *bio*250 deletion end point lies to the right of all known *c*III mutations but apparently does not delete any part of the gene for the Ea10 protein, which lies to the right of and adjacent to gene *c*III (9, 33). Thus, the *tp*1 and *tp*2*m* mutations probably lie in the rightmost segment of gene *c*III, although the possibility exists that they lie very close to the right, but outside, of *c*III.

Although *tp*1 and *tp*2*m* appear to lie within a very short segment of the *c*III gene, it was possible to isolate λ$^+$ recombinants after a mixed infection (with UV stimulation of recombination) with these two mutants. The λ$^+$ recombinants represented 0.4% of the progeny after subtraction of the reversion rate. Therefore, the *tp*1 and *tp*2*m* mutations do not cover the same region.

Tp$^+$ recombinants were recovered after λ*tp*4 infection of the defective prophage strain GK85. Along with the data from the cross with λ*imm*^{434}, this indicates that the *tp*4 mutation lies to the right of the *imm*^{434} substitution (Fig. 1; Table 4). No λ*tp*4*imm*^{21} recombinants were recovered after λ*tp*4 and λ*imm*^{21} coinfection (Table 4). These data demonstrate that the λ*tp*4 mutation lies between the right end points of the *imm*^{434} and *imm*^{21} immunity region substitutions, placing it in the *c*Y-*c*II regulatory region.

Attempts to use appropriate λ deletion phage strains with end points in the *c*Y-*c*II region (1, 29) to map the λ*tp*4 mutation more accurately were unsuccessful. This difficulty may have been due to an effect of the *nin*5 deletion, which these λΔ*N* phages must contain to replicate (4, 20), on plaque turbidity.

For reasons described below, it seems more likely that the *tp*4 mutation affects repressor synthesis through an effect on *c*II synthesis or function rather than through the *cis*-dominant *c*Y function.

Complementation for lysogeny of λ*tp*1 and λ*tp*4 mutants with λ*c*II and *c*III mutants. A simple visual assay was utilized to determine whether coinfection of λ*c*I857*tp* phage with λ*c*II or λ*c*III mutant phage would result in increased lysogeny, compared to λ*c*I857*tp*$^+$ coinfection (Fig. 2). λ*c*I857 or λ*c*I857*tp* suspensions were spotted onto a lawn of AX14 *lon* cells. A partially overlapping spot of λ*c*II2002, λ*c*Y42, or λ*c*III67 suspension at the same concentration of phage per milliliter was applied, and the plates were incubated at 37°C. At this temperature, λ*c*I857 phage produced a very clear spot with a few resistant bacterial clones present. λ*c*I857*tp*$^+$ was shown to complement λ*c*II2002 or λ*c*III67 as evidenced by a more turbid area at the intersection of the two spots. As expected, λ*c*I857 could not complement λ*c*Y42 for lysogenization, because *c*Y is a *cis*-dominant, *trans*-recessive site involved in the establishment of lysogeny and does not code for a diffusible product. Thus, the *c*Y$^+$ genotype of the infecting λ*c*I857 phage could not stimulate transcription of the *c*I$^+$ gene present in the λ*c*Y42 mutant, and lysogenization was not enhanced. The diffusible wild-type *c*II or *c*III products of λ*c*I857 could, however, complement the defects present in the λ*c*II and λ*c*III mutants.

λ*c*I857*tp*1 complemented *c*III67 or *c*II2002 phage in mixed infection and exhibited a more dramatic effect on the density of bacterial growth at the junction between the two spots than did λ*c*I857.

Of particular interest were the results found after mixed infection with λ*c*I857*tp*4 and either λ*c*II or λ*c*III. With these phages, infection with λ*c*I857*tp*4 also resulted in a much more marked increase in turbidity of the infected AX14 lawn than did λ*c*I857. The λ*tp*4 mutation, which was mapped in the *c*Y-*c*II region, apparently affects a diffusible factor which can turn on transcription of the *c*I$^+$ gene on the λ*c*II or λ*c*III phage genome. These results suggest that the λ*tp*4 mutation is within the *c*II gene, rather than an alteration of the *c*Y site, because a mutation in the latter would not be expected to act in *trans* on the *c*I$^+$ gene of a coinfecting genome.

Repressor activity after λ*tp* infection. Infection with λ*tp*1, λ*tp*2, or λ*tp*4 has been shown to result in overproduction of repressor after infection of either *lon* or *lon*$^+$ cells as compared with a λ$^+$ infection at the same apparent MOI (36). However, the MOIs in those experiments were calculated from phage titers determined on nonlysogens. Because repressor levels increase with increases in MOI (23, 36), it was important to determine whether the apparent increase in repressor levels over those found after λ$^+$ infection was an artifact of the higher actual MOIs in some of the λ*tp* infections. After correct MOI values were determined by using assays of all

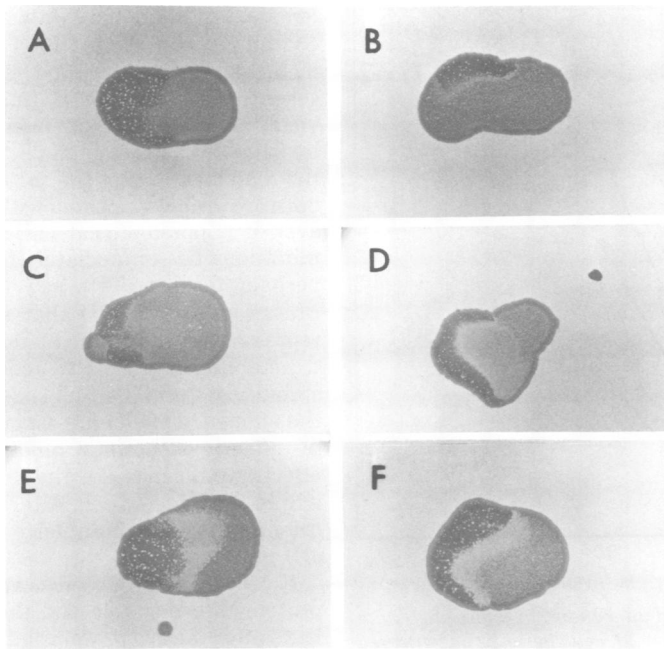

FIG. 2. *Complementation for lysogeny by λcI857tp mutants with λcII or λcIII phage. Overlapping spots of lysate were applied to a lawn of AX14 lon cells. At the left of each picture is a λcI857 phage spot, at the right is λcY42, λcII2002, or λcIII67. Plates were incubated at 37°C overnight. (A) Control which demonstrates no complementation between λcI857 and λcY42; (B) control which demonstrates complementation between λcI857 and λcII2002 as evidenced by increased turbidity at the intersection of the spots; (C) control which demonstrates complementation between λcI857 and λcIII67 as evidenced by increased turbidity at the intersection of the spots; (D) λcI857tp1 and λcII2002; (E) λcI857tp1 and λcIII67; (F) λcI857tp4 and λcII2002. Results comparable to those seen in F were found with λcI857tp4 and λcIII67. Spots of lysates were 20 μl of 10^8 phage per ml.*

titers on AB1157 (λimm^{434}) lysogens, λ repressor levels were determined after infection of 2e01c cells with either λ^+ or λ*tp*. λ*tp*1 and λ*tp*2 infection resulted in a twofold increase in repressor levels, as previously reported. λ*tp*4 infection resulted in a reproducible 40% increase in repressor levels, rather than the 100% increase previously reported (data not shown).

Phage yields after λ*tp* infection. The effect of the λ*tp* mutations on λ lytic development was examined by measuring phage yields after λ^+ or λ*tp* infection of strain 2e01c. Phage yields were reduced after infection by λ*tp* mutants (Fig. 3). λ*tp*1 and λ*tp*2*m* infection produced 10 to 35% as many progeny as λ^+; λ*tp*4 yield was 1 to 5% of λ^+ yield (in several experiments).

λ endolysin levels after λcI857*tp* infection. To test the possibility that the *tp* mutations might have a second inhibitory effect on lytic growth (in addition to inhibition which might result from overproduction of repressor), λ*c*I857*tp*1 and λ*c*I857*tp*4 phage were constructed and used to infect strain 2e01c cells at 40°C. At this temperature, the thermosensitive *c*I857 repressor would be inactivated, so that any λ*tp* effect on late lytic development, independent of the effect on repressor synthesis, could be analyzed.

After λ*c*I857*tp*1 infection, endolysin levels were only 35 to 45% of the wild-type levels over the MOI range of 1 to 5 (Fig. 4; data for MOI of 4 not shown). Moreover, endolysin synthesis was delayed by 7 to 10 min. These results strengthen the hypothesis that the *tp*1 (*c*III) gene product inhibits λ late gene product synthesis by some mechanism in addition to its stimulation of repressor synthesis.

λ*c*I857*tp*4 infections produced endolysin levels of 55 (MOI of 4) to 120% (MOI of 1) of the λ*c*I857 level. Thus, no general conclusions about the effect of the *tp*4 mutation on late gene product synthesis in the absence of repressor could be reached.

DISCUSSION

The λ*tp* mutants were isolated on the basis of their ability to form turbid plaques on *E. coli lon* cells (38). Infection with λ*tp*1 or λ*tp*2 results in twofold overproduction of λ repressor in both

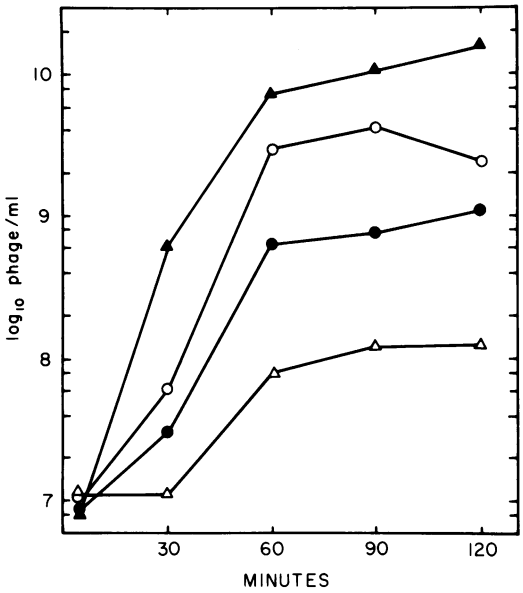

FIG. 3. *Phage yield after λ⁺ or λtp infection. Strain 2e01c was grown to 3 × 10⁸ cells per ml, concentrated 10-fold, and infected with λ⁺ (▲), λtp1 (○), λtp2m (●), or λtp4 (△) at an MOI of approximately 3. The cultures were diluted 10⁻³ and incubated at 37°C. MOIs were 3.0 (λ⁺), 2.7 (λtp1), 2.6 (λtp2m), and 3.0 (λtp4). Similar results were obtained after infection at an MOI of 4.*

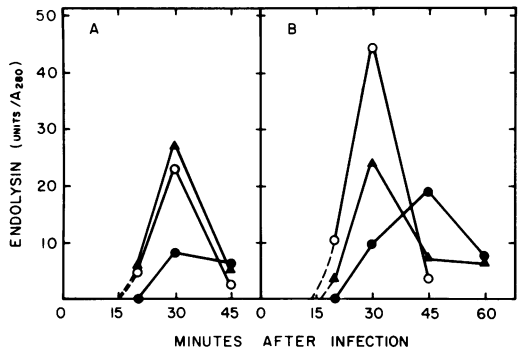

FIG. 4. *Effect of the tp mutations on endolysin synthesis in the absence of functional cI repressor. Cells of strain 2e01c were grown at 37°C to 3 × 10⁸ cells per ml, concentrated 10-fold, and infected with λcI857 (○), λcI857tp1 (●), or λcI857tp4 (▲) at an MOI of approximately 1 (A) or 4 to 5 (B). Cultures were then diluted into YET broth prewarmed at 40°C and incubated. (A) λcI857 MOI was 0.9, λcI857tp1 MOI was 1.0, and λcI857tp4 MOI was 1.0; (B) λcI857 MOI was 4.8, λcI857tp1 MOI was 5.0, and λcI857tp4 MOI was 4.1. For λcI857tp1 infections, results similar to the data of A were obtained if the temperature was 37°C rather than 40°C, and results similar to the data of B were obtained when the MOI was 4 rather than 5.*

lon and *lon*⁺ cells, but established monolysogens contain normal levels (36). Thus, the mutations in these strains affect the establishment of lysogeny only and not its maintenance. Phage yields after λ*tp*1 or λ*tp*2 infection were reduced by 65 to 90%. These findings are consistent with the map locations of the *tp*1 and *tp*2 mutations: both are probably located within gene *c*III. However, it is possible that either or both of these mutations lies immediately to the right of *c*III, perhaps resulting in a new leftward promoter. Mutations within *c*III that result in increased repressor synthesis might do so by enhancing the stability of the mRNA coding for *c*III or the stability of the *c*III protein itself, or by increasing the efficiency of *c*III messenger translation (e.g., by affecting ribosome binding). Any of these effects would increase the level of *c*III protein within the infected cell. Alternatively, the *tp*1 or *tp*2 mutation might enhance the activity of the *c*III product.

It has been proposed that the *c*II and *c*III proteins are unstable and their activity diminishes after the *cro* protein shuts off transcription of these genes (23). In this manner, the *cro* product causes a termination of *c*II-*c*III-mediated *cI* (repressor) transcription. If the *tp* mutations resulted in an increased stability of the mRNA or protein products of gene *c*III, or in increased synthesis of the *c*III protein, then the effect of the *cro* product on the termination of repressor synthesis would be somewhat delayed. A short delay in shutoff of repressor synthesis was indeed observed after infection by both λ*tp*1 and λ*tp*2 (36).

The λ*tp*4 mutation, which also affects repressor level specifically during establishment of immunity, mapped in the *c*Y-*c*II regulatory region. The ability of λ*c*I857*tp*4 to complement a λ*c*II phage for lysogenization at 37°C to a more marked degree than could λ*c*I857 suggests that *tp*4 affects a diffusible product involved in repressor synthesis (*c*II) rather than a *trans*-recessive site such as *c*Y. The *tp*4 mutation could affect repressor synthesis through an alteration of *c*II protein levels or activity, in manners analogous to those proposed above for *tp*1 and *tp*2. Thus, the *tp*4 mutation might be *cis*-acting to affect the diffusible *c*II protein (e.g., by creating a new promoter). DNA sequence analysis of the *tp*4 mutation, such as that done recently for genes and markers in the *cro*-*c*Y-*c*II region (16, 25–28, 31), might distinguish between these possibilities.

It might be imagined that the *tp* mutants would plate on wild-type hosts with low efficiency because of repressor overproduction. However, the *tp* mutants synthesize approxi-

mately the same, or even lower, repressor levels in *lon* hosts as does λ^+ in lon^+ hosts (36). The fact that λ*tp* mutants plate with about equal frequency on both lon^+ and *lon* hosts made it surprising that λ*tp*1, λ*tp*2, and λ*tp*4 plate more efficiently on λimm^{434} heteroimmune lysogens than on nonlysogens. These results suggest that the *tp* mutations inhibit lytic development, and hence, plaque formation, by some mechanism in addition to their effect on λ repressor synthesis after infection. Such inhibition might be relieved by *trans*-activation of λ late genes present in the prophage (35). The *tp* mutants plated less efficiently on λimm^{21} lysogens than on λimm^{434} lysogens; perhaps late functions are *trans* activated to differing extents from the λimm^{21} and λimm^{434} prophages.

It has been suggested (3, 18) that the λ genes involved in the initial stimulation of λ*c*I repressor transcription, *c*II and *c*III, and site *c*Y might also be directly involved in delaying λ late gene product synthesis. This direct effect would be in addition to the repression caused by the *c*I gene product and might be mediated by interference with rightward transcription of *cro* and the λ late genes (30). The λ*tp* mutations, which appear to increase the activity, stability, or synthesis of *c*III (λ*tp*1, *tp*2) and *c*II (λ*tp*4), might also affect this proposed aspect of *c*II-*c*III function.

This possibility was tested by constructing λ*c*I857*tp*1 and λ*c*I857*tp*4 phage and measuring the endolysin levels achieved after infection at 40°C, at which temperature the *c*I857 repressor is inactive. λ endolysin is the product of gene *R*, located in the right arm of the λ prophage map, and is involved in cell lysis late in the lytic cycle (8, 34). λ*c*I857*tp*1 infection clearly resulted in lowered and delayed endolysin production, compared with λ*c*I857tp^+. Inasmuch as *tp*1 mapped in the *c*III region, these results strengthen the hypothesis that the *c*III product acts directly on the synthesis of λ late gene functions in addition to its effect on *c*I transcription.

λ*c*I857*tp*4 infection resulted in endolysin levels ranging from 55 to 120% of λ*c*I857tp^+-mediated levels. The mechanism(s) of *tp*4 action on λ lytic development, therefore, remains largely unresolved. λ*tp*4 infection resulted in considerably reduced phage yields (1 to 5%), lower than the levels observed after either λ*tp*1 or λ*tp*2 infection. Yet, λ*tp*4 infection increased repressor synthesis by only 40%, compared with the 100% increase after λ*tp*1 or λ*tp*2 infection. Furthermore, λ*tp*4 had the lowest efficiency of plating of the *tp* mutants on nonlysogens compared with plating efficiency on a λimm^{434} heteroimmune lysogen. Thus, it is likely that the *tp*4 mutation affects λ lytic development by a second, as yet undefined, mechanism in addition to its effect on repressor synthesis after infection.

The *tp*4 mutation (thought to affect the diffusible *c*II product) differs from the *cin*-1 mutation which increases lysogenization after infection. *cin*-1 maps in the *c*Y region and is *cis* dominant, *trans* recessive in activity (26, 39). *tp*4 differs also from the *sar* mutation, which maps in the *ori* region between *c*II and *O*, depresses repressor synthesis by a factor of 2 after λ*sar* infection, and suppresses the *c*Y mutation effect on repressor synthesis after λ*c*Y *sar* infection (11).

ACKNOWLEDGMENTS

We thank W. G. Haldenwang for helpful discussions and help with the repressor assays, and D. Kaiser for hospitality while J. R. W. learned to make λ crosses in his lab.

C. L. T. was a National Science Foundation Predoctoral Fellow and was supported in part by Public Health Service Training Grant 1 T32 GM07126 from the National Institute of General Medical Sciences. This work was supported by Public Health Service grant AI 08286 from the National Institute of Allergy and Infectious Diseases and in part by American Cancer Society grant NP169.

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