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A New Host Gene (*gro*PC) Necessary for Lambda DNA Replication

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Summary. The isolation of a bacterial mutation in a gene, designated *gro*PC, which affects the growth of phages lambda and P2 is described. Lambda replication is severely limited in the strain, and some lambda π mutations, which map in (or near) the *P* gene, allow growth. The *gro* mutation, *gro*PC259, is recessive to wild type and maps between threonine (*thr*) and diaminopimelate (*dap*B) on the *E. coli* chromosome. The possibility that the *gro*PC gene is concerned with host DNA replication is discussed.

Introduction

The isolation of bacterial mutants unable to support virus growth is a powerful approach to defining host functions necessary for viral development. The further isolation of virus mutants which can grow on the bacterial mutants provides information about the stage of virus development involved and the virus gene(s) involved.

Bacterial mutants unable to support lambda development have been intensively studied by Georgopoulos and coworkers. Three classes of *gro* mutants, *gro*N (Georgopoulos, 1971; Pironio and Ghysen, 1970), *gro*P (Georgopoulos and Herskowitz, 1971) and *gro*E (Georgopoulus et al., 1973) have been described. In each class a particular step in lambda development is blocked, and the designation (e.g., *gro*P) indicates the lambda gene involved (*P*). For the *gro*P example, lambda is unable to replicate normally, but certain lambda mutants, called λ π, have a mutation in the *P* gene which compensates for the *gro*P block, allowing replication.

The isolation of *gro* strains is also useful in defining host genes which may also be essential for bacterial growth. For example, Sunshine and Sauer (1975) isolated a *gro* strain unable to support phage P2 development. The *gro* mutation of Sunshine and Sauer has been shown to be in the gene for the alpha subunit of RNA polymerase (Fujiki et al., 1976), for which no mutations had previously been described.

Because *gro* mutations by definition block virus growth but not host growth, *gro* mutations are likely to be rather subtle changes which affect host functions with respect to interaction with virus functions without grossly impairing the cellular role of the host functions. Hence a *gro* mutation which affects growth of one virus may not affect another virus even though the host protein is required by both. This is the case with *gro*P mutations, which are a subclass of *dna*B mutations (D'Ari et al., 1975). Although both lambda (Fangman and Novick, 1968; Fangman and Feiss, 1969) and P2 (Bowden et al., 1975; Sunshine et al., 1975) require the *dna*B gene product for replication, *gro*P strains allow P2 to grow. In fact there is not a great overlap between bacterial strains that are *gro* for lambda and *gro* for P2. The P2 *gro* strain with altered RNA polymerase supports lambda growth and *gro*P, N, and E strains support P2 (Sunshine, unpublished).

We decided to search for bacterial mutants that were *gro* for both lambda and P2, to examine the possibility that the co-selection might reveal rare classes of *gro* mutants not yet found by single selection. This report concerns such a strain, MF634, which contains a mutation in a bacterial gene, called *gro*PC, which affects lambda and P2. The interaction of *gro*PC with lambda is described.

During the course of our experiments, we became aware that one of the *gro*P strains described by Georgopoulos and Herskowitz (1971) contains a *gro*P mutation which is likely to be in the same gene as our *gro*PC mutation. The mutation of Georgopoulos and

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Table 1

a) Bacteriophage strains

Strain	Remarks	Source/Reference
λ*c*I60		I. Herskowitz; Georgopoulos and Herskowitz (1971)
λ*c*I60 πA1		I. Herskowitz; Georgopoulos and Herskowitz (1971)
λ*c*I60 πB1		I. Herskowitz; Georgopoulos and Herskowitz (1971)
21*gp*		D. Kaiser
T4		Our collection
T7		Our collection
P1kc		Our collection
P1 Cml *clr*100		Rosner (1972)
φ80hy21-5	*imm*80*h*λ	N. Franklin
Mu1		M. Howe
λ*c*I857		Our collection
λ*Pam* strains		Our collection
P2		Our collection; Bertani and Bertani (1971)
P2*vir*1	unable to establish lysogeny	Our collection; Bertani and Bertani (1971)
λ*imm*434 *c*I *bio*1	unable to establish lysogeny, growth not blocked by P2	Our collection

b) Bacterial strains

Strain	Relevant properties[a]	Source/Reference
C600	*thr leu thi sup*E	Bachmann (1972)
MF634	C600 *gro*PC259	This work
C600-15	C600 *gro*PA15	C. Georgopoulos; Georgopoulos and Herskowitz (1971)
GR7	*sup*°, *gro*PB558	I. Herskowitz; Georgopoulos and Herskowitz (1971)
F'101/AB2463	*thr*$^+$ *leu*$^+$/*thr leu thi* his *arg*E *pro*A *ara lac gal xyl mtl rec*A13 *tsx rps*L	B. Bachmann
NY179	F'101 *dna*C2/*leu arg*G *met lac gal rec*A56 *rps*L	J. Wechsler; Wechsler (1973)
NY180	F'101 *dnaC*$^+$ *ser*B/*leu arg*G *met lac gal rec*A56 *rps*L	J. Wechsler; Wechsler (1973)
SA439	*chl*A-prophage deletion strain see Figure 2	S. Adhya; Stevens et al. (1971)
SA431	*chlA*-prophage deletion strain see Figure 2	S. Adhya; Stevens et al. (1971)
MF687	*mut*D *thr leu thi his arg lac xyl mtl ara rps*L	Degnen et al. (1974)
AT2459	*thi ser*B22 *rel*1	B. Bachman
AT999	*thi rel*1 *dap*B17 (Mu1)	B. Bachman
HfrH	order of transfer *thr leu lac*	Our collection
GR756	C600 *gro*PC756	C. Georgopoulos

[a] Genetic designations conform to the nomenclature of Bachmann et al. (1976); *rps*L indicates streptomycin resistance

Herskowitz was named *gro*PAB756; it has been renamed *gro*PC756 by Georgopoulos (see accompanying report). We also present here studies on the relationship of our *gro*PC mutation to *gro*PC756.

Materials and Methods

Phage and Bacterial Strains. These are listed in Table 1.

Media. Tryptone broth (TB), TB soft agar and TB agar were as described by Campbell (1961) except each was supplemented with 0.01 M $MgSO_4$ and for certain experiments NaCl (normally 0.5%) was omitted. LB broth and LB agar were as described by Bertani (1951). The synthetic medium was that of Davis and Mingioli (1950), appropriately supplemented. For density labeling, heavy M medium (Stahl and Stahl, 1971) with $^{15}NH_4Cl$ and [^{13}C] glucose (52 atom percent, Merck) was used, supplemented with [^{12}C] threonine, leucine and thiamine for auxotrophic requirements.

Isolation of MF634. An overnight culture of C600 in 7 ml LB broth containing 0.005 M $CaCl_2$, 0.01 M $MgSO_4$, and 0.2% maltose was spun and resuspended to 1×10^8 cells/ml in 0.01 M $MgSO_4$. The cells were treated with an ultraviolet light dose of 300 ergs/mm^2 and then transferred to 18 ml of the growth medium and allowed to grow in the dark for 3 h at 30° C. 2 ml of these cells at approximately 1×10^8/ml were coinfected with λ*imm*434 *cI bio*1[1] at a multiplicity of 4 phage/cell and P2*vir*1 at a multiplicity of 16 phage/cell, and absorption was allowed for 10 min at 37° C. Serial 10 fold dilutions were made, and aliquots were spread on LA plates. After overnight incubation at 37° C, colonies were picked into nylon microculture containers (Elesa, Milano) and streaked on hard LB agar plates. Reisolated colonies were picked into LB broth, allowed to grow at 37° C for 18 h, and replicated to LB agar plates seeded with 5×10^8 P2*vir*1 and to tryptone agar plates seeded with 5×10^7 λ*c*I60 and incubated overnight at 37° C. Cultures corre-

[1] P2 interferes with the growth of λ unless the P2 is *old*$^-$ or the λ is *spi*$^-$ (Lindahl et al., 1970). Hence, we have used phage λ*imm*434 *c*I *bio*1 which exhibits the *spi*$^-$ phenotype

sponding to spots not lysed by both λ and P2 were retested for ability to plate and adsorb λ and P2.

Density Transfer. C600(λ*c*I857) was grown and heat induced in heavy M medium. The resulting lysate was dialyzed and used to infect C600 of MF634 (grown up in TB+ 0.2% maltose at 37 °C and resuspended in 0.01 M $MgSO_4$) at a multiplicity of 5 phage/cell. After 15 min at 23° C, the infected cells were diluted into TB and aerated at 37° C for 90 min. Each resulting lysate was mixed with a stock CsCl solution to give 5.0 ml of n=1.3830. This was centrifuged in the Spinco SW 50.1 rotor for 20 h at 29 K revs/min at 20° C. The resulting gradients were analyzed by collecting drops (about 100) from the bottom into tubes which were then titered.

Mutagenesis. MF687 was grown to 1×10^8/ml in LB broth plus 0.2% maltose at 37° C, infected with λ*c*I60 at a multiplicity of 5 and aerated at 37° C until lysis. MF687 contains a *mut*D mutator mutation, described by Degnen et al. (1974), which results in strong mutagenesis of phage lambda under the conditions employed here. A stock of λ^+ contained 2% clear plaque mutants after a growth cycle on MF687.

Marker Rescue. SA431 and SA439 were grown in TB plus 1 μg/ml biotin and 0.2% maltose to 1×10^8 cells/ml. The cells were resuspended in 0.01 M $MgSO_4$ at 5×10^8/ml. The cells were infected at a multiplicity of 2 with λ*c*I60 π*C*8 phage which had previously been irradiated with 1000 ergs/mm^2 of ultraviolet light. After 15 min for adsorption the infected cells were diluted 100 fold into 0.01 M $MgSO_4$, irradiated with an additional 100 ergs/mm^2, diluted 100 fold into TB+biotin and shaken in the dark for 70 min at 37° C. The lysate was plated at 34° C on C600, 3 to 5% of the progeny were *c*I857 recombinants that formed turbid plaques; these were picked and tested for the π character by spotting on MF634.

Phage Growth Experiments. C600 and MF634 were grown in TB plus maltose at 37° C to 5×10^8/ml and resuspended in 0.01 M $MgSO_4$. Phage were added at 0.1 phage/cell, and adsorption was for 15 min at 23° C. Anti-lambda serum was added to inactivate unabsorbed phage for 10 min at 23° C. Infected cells were pelleted, resuspended and diluted to ca. 5×10^4/ml in TB. Infectious centers were measured by titering immediately and phage yields measured after 70 min at 37° C.

Acridine Orange Curing. Curing using 50 μg/ml acridine orange was performed as described by Miller (1972).

Incorporation Studies. To measure DNA synthesis, cells were grown to ca. 1×10^8/ml in TB lacking NaCl with 250 μg/ml deoxyadenosine and 0.5 μCi/ml [^{14}C] or [^{3}H]-thymidine at 30° C. The culture was shifted to 44.5 C and incubation continued. For protein synthesis [^{14}C]-labeled amino acids at 0.5 μCi/ml were used in place of deoxyadenosine and thymidine. Turbidity was followed with a Klett colorimeter and incorporation measured by trichloroacetic acid precipitation of 1 ml samples which were filtered and counted.

Bacterial Crosses. Bacterial crosses were performed as described by Miller (1972).

Transduction Experiments. P1 transductions were performed as described by Sunshine and Kelly (1967). The P1 transducing lysate used in the experiments described in Table 4 was prepared by heat induction of MF634 lysogenic for a variant of P1 Cml *clr*100. We found that MF634 lysogens of P1 Cml *clr*100 lysed poorly upon induction and that very few phage were released (1 viable phage/10^4 induced cells). MF634 was lysogenized with one of the phages released from MF634 (P1 Cml *clr*100). The resulting lysogen was found to lyse normally upon induction and a normal yield (about 50 phage/induced cell) was obtained. This suggests that a variant of P1 Cml *clr*100 had been recovered from MF634 (P1 Cml *clr*100). We are currently studying this matter.

Results

1. Studies of MF634

Isolation and General Properties of MF634. Among 300 survivors of co-infection by λ*imm*434 *c*I *bio*1 and P2*vir*1, one called MF634 adsorbed λ and P2 normally but did not allow plaque formation by lambda (the efficiency of plating relative to the parent C600 was less than 10^{-4}), and plaque formation by P2*vir*1 was reduced (efficiency of ca. 0.2 or less). When this *gro* mutation was transferred to other strains by P1 transduction, the effect on P2 remained the same (e.o.p. of 0.17 for P2*vir*1 and 0.026 for P2$^+$). Additional phages unable to grow on MF634 include 21 and φ80hy 21-5 (C600 and MF634 are *ton*B and are unable to adsorb φ80, so hybrid phage with the capsid genes of lambda was used). Phages T4, T7 and Mu1 plate normally on MF634. Of several lambda mutants tested for growth, λπA1 and λπB1 were able to plate with an efficiency of one.

Lambda Replication is Severely Limited in MF634. The finding that λπ strains are able to grow in MF634 suggested that the *gro* mutation of MF634 interfered with lambda replication. Accordingly, lambda replication in MF634 was studied by infection with [^{15}N, ^{13}C]-labeled λ*c*I857. Lysates obtained from infection of MF634 and its parent C600 (in light medium) were banded to equilibrium in CsCl density gradients and analyzed by titering fractions. The results are shown in Figure 1. The left panel shows the profile of the

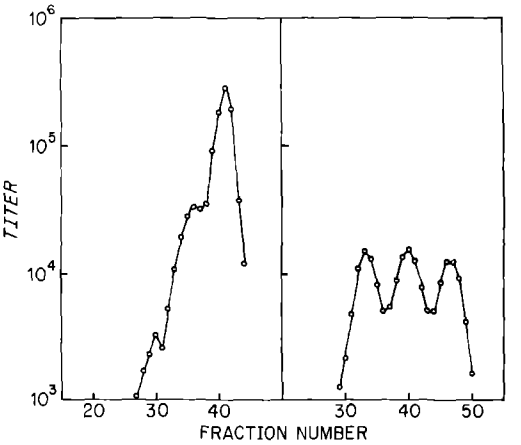

Fig. 1. CsCl density gradient analysis of phage lysates. Cells were infected with [^{15}N, ^{13}C]-labeled λ*c*I857 and shaken for 90 min at 37° C. Left panel: infection of C600; right panel: infection of MF634

Table 2. Growth ability of λπ strains[a]

Host	λ	λπA	λπC	λπB	Mutant frequency[b]
*gro*PA	−	+	+	+	2×10^{-3}
*gro*PC	−	−	+[c]	+[c]	4.2×10^{-5}
*gro*PB	−	−	−	+	2.7×10^{-5}

[a] Plus indicates an efficiency of plating near one, relative to C600, the parent of MF634, minus indicates the efficiency is $<10^{-4}$

[b] The λπ strains were isolated from a stock of λ*c*I60 that had been grown one cycle on MF687, a *mut*D host. The frequency is the titer of π mutants relative to the titer of the mutagenized stock on C600

[c] The plaques are quite small (see Table 5)

Table 3. Effect of the *gro*PC mutation on lambda growth

	Efficiency of[a] transmission	Burst[b] size
C600		
λ*c*I60	0.60	250
λ*c*I60 πC8	0.62	173
MF634		
λ*c*I60	0.0008	1.2
λ*c*I60 πC8	0.30	2.0

[a] Fraction of infected cells (at a multiplicity of 0.1 phage/cell) releasing progeny. Average of two experiments

[b] Final phage yield divided by the number of cells releasing progeny phages

C600 lysate; most of the progeny phages bear fully light chromosomes, an index of extensive replication, and the other two peaks are phages with fully heavy and hybrid chromosomes. The MF634 lysate profile (right panel) consists of roughly equal amounts of phages bearing unreplicated, hybrid, and fully light progeny chromosomes. This indicates that replication is severely limited but not completely blocked in MF634.

Some λπ Mutations (πC) Allow Growth in MF634 (a groPC mutant). The observation that λπ strains are able to grow on MF634 suggested that lambda mutants able to grow on MF634 would be π mutants. We isolated collections of mutants able to grow on *gro*PA, *gro*PB and MF634 strains. Examination of these collections showed MF634 to be a new type of *gro*P mutant, designated *gro*PC259, able to support growth of λπB mutants and a new class, λπC. The growth pattern is presented in Table 2.

All (10/10) λπB strains isolated on a *gro*PB strain, *gro*PB558, were able to grow on the *gro*PA15 and *gro*PC259 strains. Of 12 strains isolated on MF634, 10 were classified as πC strains (see Table 2), and the remaining 2 were λπB strains. Of 12 strains isolated on *gro*PA15, none was able to form plaques on MF634; but λπA1 of Georgopoulos and Herskowitz (1971) is able to plate on MF634. No *P* amber strains tested (80, 3, 902, 901) were able to grow on *gro*PC259. In summary, there is a hierarchy of π mutations; πB mutations can be regarded a subset of πC mutations which are in turn a subset of πA mutations.

We performed infection experiments to determine the effect of the *gro*PC259 mutation on wild type lambda (λ*c*I60) growth and the effect of a πC mutation on growth in the *gro*PC host. The results, presented in Table 3, show that the *gro*PC259 mutation greatly reduces the number of infected cells releasing any progeny, and furthermore greatly reduces the burst size of those cells which do release phage. The effect of the πC8 mutation is to increase 300 fold the fraction of infected cells releasing progeny; the burst size is still quite low. This is consistent with the plating behavior of the πC8 phage: a high efficiency of plating and greatly reduced plaque size.

Mapping a πC Mutation. The fact that lambda mutants which grow on the *gro*PC259 strain are π mutants strongly suggested that the πC mutations would map in the *P* gene of lambda, as do πA and πB mutations (Georgopoulos and Herskowitz, 1971). Accordingly, we asked whether the wild type allele of the πC8 mutation could be rescued from prophage deletion strains which had (SA431) or did not have (SA439) the lambda *P* gene. These strains also are

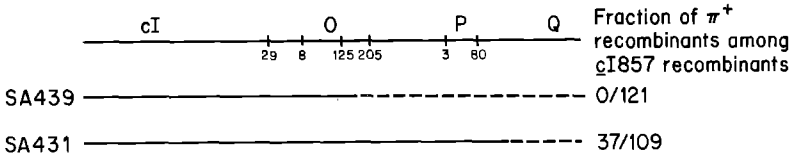

Fig. 2. Marker rescue from deletion prophages. The top line is the order of selected markers in the *c*I to *Q* region of the lambda map, distances are arbitrary. Lower lines show remaining prophage material (solid lines) and deleted material (broken lines) of deletion strains SA439 and SA431 (taken from Stevens et al., 1971). The *c*I genes of the prophages carry the *c*I857 mutation. These strains were infected with λ*c*I60 πC8 with UV-stimulation of recombination. The lysates were plated at 34° C and *c*I857 recombinant phages (turbid plaques) were further tested for the π allele. The frequency of rescue of π^+ along with *c*I857 is given in the right column

Table 4. Mapping of *gro*PC259 by P1 transduction

Donor: MF634 = *ser*$^+$ *dap*$^+$ *thr*$^-$ *leu*$^-$ *gro*PC259

Unselected markers	A) Recipient: AT2459 = *ser*B *thr*$^+$ *gro*$^+$ selection = *ser*$^+$		B) Recipient: AT999 = *dap*B *thr*$^+$ *leu*$^+$ *gro*$^+$ selection = *dap*$^+$	
	Observed frequency (%)	Expected frequency (%)[a]	Observed frequency (%)	Expected frequency (%)[a]
*gro*PC259 *thr*$^-$	25	14	27	14
*gro*PC259 *thr*$^+$	4	15	18	31
gro$^+$ *thr*$^-$	23	34	4	17
gro$^+$ *thr*$^+$	48	37	51	38
	A) 211 transductants tested		B) 198 transductants tested	
	CT[b] *gro*PC259 = 29%		CT[b] *gro*PC259 = 45%	
	CT *thr*$^-$		CT *thr*$^-$ = 31%	

[a] Expected CT frequencies were calculated from the actual CT frequencies obtained for the two unselected markers taken separately

[b] CT, cotransduction

*c*I857, and so we infected with λ*c*I60 πC8 and determined the frequency of π$^+$ recombinants among recombinants carrying *c*I857 (at 34° C *c*I60 parental phages make clear plaques, *c*I857 recombinants make turbid plaques). As noted in Figure 2 rescue of π$^+$ from SA431 was observed, and no rescue was found from SA439. This maps πC8 to the prophage DNA segment in SA431 and absent in SA439; this segment includes the *P* gene and some neighboring sequences. We conclude that the πC8 mutation is in all likelihood in the *P* gene, though the formal possibilities remain that it could map in the rightmost part of *O* or between *P* and *Q*.

Mapping the groPC259 Mutation. The approximate location of *gro*PC259 was determined by bacterial matings between HfrH and MF634. These experiments (data not shown) placed the *gro*PC259 gene very near the *thr-leu* region on the *E. coli* map. Hence, P1 grown on HfrH was used to transduce MF634 to *thr*$^+$ and *leu*$^+$. Linkage of *gro*$^+$ to *thr*$^+$ was observed: 56% (76/135) of the *thr*$^+$ transductants were found to be *gro*$^+$, whereas, only 1% (1/98) of the *leu*$^+$ transductants were *gro*$^+$. In order to map more precisely the *gro*PC259 mutation relative to *thr*, the transductions listed in Table 4 were performed; selection was for *ser*B$^+$ in Transduction A and for *dap*B$^+$ in Transduction B. These markers were chosen because *ser*B is counter clockwise from *thr* and *dap*B is clockwise from *thr* (Bachman et al., 1976). In Transduction A, both *gro*PC259 and *thr*$^-$ were contransducible with ser$^+$ selection, with *thr*$^-$ being closer than *gro*PC259. The sequence *ser-thr-gro*PC259 is indicated by (a) the observed cotransduction frequencies for the two unselected markers which differ from the values expected for independence of transfer (i.e., the unselected markers being on opposite sides of the selected marker), and (b) the least frequent transduction class, *gro*PC259 *thr*$^+$, which is interpreted as the quadruple exchange class. With selection for *dap*B$^+$ (Transduction B), *gro*PC259 and *thr*$^-$ were cotransducible, with *gro*PC259 being closer to *dap*B than *thr*$^-$. The sequence *thr-gro*PC259-*dap*B is indicated by (a) the lack of independence of transfer of the unselected markers, and (b) the least frequent transduction class, *gro*$^+$ *thr*$^-$, which is interpreted as the quadruple exchange class. In Transduction B, *leu*$^-$ was also cotransducible with *dap*B$^+$ selection (data not shown). The observed cotransduction frequencies for *gro*PC259 and *leu*$^-$ as unselected markers were similar to the expected values indicating independence of transfer. This is compatible with *gro*PC259 and *leu*$^-$ being on opposite sides of *dap*B. The data presented in Table 4 best fit the sequence *ser*B-*thr-gro*PC259-*dap*B-*leu* as shown in Figure 3. Subjecting the cotransfer frequencies shown in Figure 3 to the Wu formula (Wu, 1966) to compute map intervals in minutes from gene cotransduction frequency data, we place *gro*PC259 between 0.25 and 0.35 min clockwise of the *thr* marker on the *E. coli* genetic map.

The groPC259 Mutation is Recessive. In order to examine the complementation behavior of *gro*PC259, we constructed *gro*$^+$/*gro*PC259 heterozygotes by

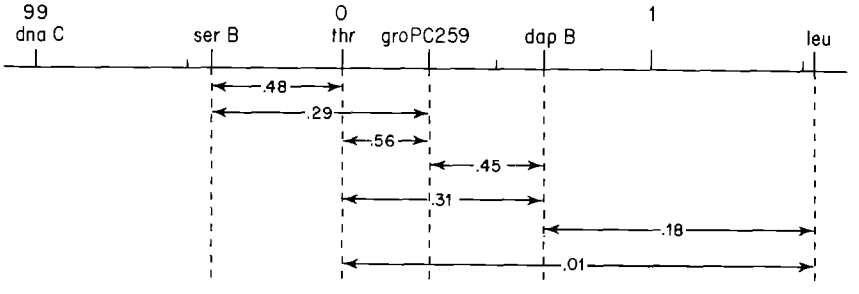

Fig. 3. Detailed genetic map of the *gro*PC region of the *E. coli* map. The order of the markers follows Bachmann et al. (1976). *gro*PC259 is placed on the map according to the results presented in Table 4. Numerical values given are cotransduction frequencies (Table 4 and data not presented)

transferring the F'101 episome into MF634. F'101 carries a segment of the *E. coli* chromosome that includes the *thr* to *leu* region. MF634 is auxotrophic for threonine and leucine, so we selected MF634/F'101 isolated as thr^+ leu^+ progeny from a cross of MF634 with AB2463/F'101 (donor cells were selected against by omitting the required amino acids arginine, histidine and proline). Since the *gro*PC259 mutation maps between *thr* and *leu*, it was expected that the thr^+ leu^+ isolates would be gro^+/*gro*PC259 heterozygotes. Five independent heterozygotes were purified and tested for the ability of support lambda growth at 37° C. All five allowed lambda to plate with an efficiency of 1, showing that the *gro*PC259 mutation is recessive.

To verify that heterozygotes had actually been obtained, the MF634/F'101 strains were subjected to acridine orange curing. About 30% of the cells from each of the 5 acridine orange treated cultures were gro^-, showing that the *gro*PC259 allele was present in all five strains.

We also confirmed that *gro*BC259 was distinct from *dna*C (see Figure 3), a gene which may also affect lambda development (Carl, 1970). We prepared 5 independent MF634/F'101*dna*C2 heterozygotes and found that λ^+ could plate with an efficiency of 1 on them at 41° C, a temperature at which the *dna*C2 product is thermally inactivated (Carl, 1970). Barring interallelic complementation, the *gro*PC^+ gene product supplied by F'101 must not be the *dna*C gene product.

Physiological Studies of groPC259. The *gro*PA and *gro*PB mutations alter the host *dna*B gene product, rendering DNA synthesis and growth thermosensitive. Accordingly, we examined these properties in *gro*PC259. The *gro*PC259 strain is thermosensitive, but only under severe conditions: at 44.5° C in TB lacking NaCl. These are conditions under which the parental strain C600 grows at a reduced rate. The salt effect is not understood, but certain thermosensitive *dna*B strains are rendered thermostable by addition of salt (Ogawa, 1975). When a culture of *gro*PC259 is shifted to the restrictive temperature, the mass (turbidity) of the culture increases 2 to 3 fold before a plateau is reached. Cell division ceases more rapidly than growth, because the cells become distinctly elongated. Following a shift to non-permissive conditions DNA synthesis, measured by ^{14}C or ^{3}H-thymidine incorporation, continued at a declining rate until the net incorporation reached a plateau of 170% (average of three determinations) of the amount at the time of the shift, while the mass increased an average of 290%. Incorporation of ^{14}C-amino acids, however, closely paralleled the mass increase. The results are consistent with the notion that the temperature sensitivity of MF634 is due to a defect in DNA synthesis, but obviously further studies are required.

2. Comparison of the groPC259 and groPC756 Mutations

The groPC259 and groPC756 Mutations are Closely Linked. We have performed reciprocal two-factor crosses, by P1 transduction, which indicate that the *gro*PC259 and *gro*PC756 mutations are very closely linked.

In the first cross, a thr^+ revertant of MF634 was used as the donor. A P1 Cml *clr*100 lysogen (the variant described in Materials and Methods was used) was induced to prepare the P1 lysate. The lysate was used to transduce GR756 to thr^+. Fifty thr^+ transductants were scored for the *gro* character. Only one of the 50 transductants was gro^+, 32 were scored as *gro*PC259 cotransductants and 17 had retained the *gro*PC756 mutation (the *gro*PC259 and *gro*PC756 mutations are distinguishable, as described below).

For the second cross a lysate of a thr^+ derivative of GR756 (P1 Cml *clr*100) was used to transduce MF634 to thr^+. Recombinants were tested for the *gro* character: of 122 tested, one was gro^+, 82 were *gro*PC756, and the remaining 39 had retained the *gro*PC259 marker. Thus, the two *gro*PC markers behave as closely linked markers.

Subclasses of λπC Strains. We find little correlation between λπC strains which grow on *gro*PC259 and those which grow on *gro*PC756. Table 5 presents the plating behavior of a number of the λπC strains. Results for λπB1 which grows on both *gro*PC strains are included for comparison.

None of our 10 independent λπC strains, isolated on MF634 (λπC8 is the example used), is able to form plaques on *gro*PC756 bacteria. Similarly, none

Table 5. Efficiency of plating of λπ strains on *gro*PC hosts

Phage[a]	C600	MF634 (*gro*PC259)	GA756 (*gro*PC756)
λπA1	1.0 (2.0)[b]	0.3 (0.3)	4×10^{-2} (0.2)
λπC22, λπC31	1.0 (1.8)	$<10^{-5}$	0.6 (1.5)
λπC8 and 9 others	1.0 (2.0)	0.7 (0.3)	$<10^{-5}$
λπB1	1.0 (2.0)	0.4 (0.3)	0.7 (1.2)

[a] All strains carry the *c*I60 mutation. λπA1 is the isolate of Georgopoulos and Herskowitz (1971); this strain is classified a πC strain according to Table 2

[b] Numbers in parentheses are plaque diameters in millimeters

of 8 λπC strains isolated on GR756 was able to grow on MF634.

Examination of the 12 λπ strains isolated on the *gro*PA15 strain showed two to be λπC strains (22 and 31) which grow only on the *gro*PC756 strain, forming large plaques.

Finally, the λπA1 strain of Georgopoulus and Herskowitz (1971) forms small plaques on the two *gro*PC strains, making it the only πC strain (of our limited sample) able to grow on both *gro*PC strains.

Discussion

Since *gro*PA and *gro*PB mutations represent a subclass of *dna*B mutations (Georgopoulos and Herskowitz, 1971; D'Ari et al., 1975), it seems likely that the *gro*PC gene product is also involved in DNA replication. Our incorporation studies, though not definitive, support this notion. Among previously characterized *dna* mutations of *E. coli*, lambda replication is dependent on all host elongation functions (*dna*B, Fangman and Feiss, 1969; *dna*E, Shizuya and Richardson, 1974; *dna*G, Gross, 1972; *dna*Z, Walker, et al., 1976), and is not dependent on initiation functions (*dna*A, Hirota et al., 1970; *dna*C, Carl, 1970). The inability of λ to replicate normally in MF634 points to elongation as the function of the *gro*PC gene product. Definitive identification of the *gro*PC gene as a *dna* gene must await either the isolation of mutants with a lower shutoff temperature or biochemical identification of the gene product. With respect to the latter approach, several proteins necessary for the *in vitro* replication of ϕX174 DNA have not yet been defined genetically (Shekman et al., 1974).

Two general hypotheses were considered by Georgopoulos and Herskowitz (1971) as explanations for the *gro*P-λπ system. Our data do not discriminate between the two possibilities, but instead add a third component, *gro*PC, to the models.

(a) Specific Interaction. A viral *P* protein : host *dna*B protein complex may form as an essential component in lambda DNA replication. Then *gro*PA and B mutations would be changes in the *dna*B protein that block the specific interaction with *P* protein while allowing the *dna*B protein to function in host replication. The πA and πB mutations would result in changes in the *P* protein which would restore the ability to complex with the *dna*B protein. Our results add a third element, the *gro*PC gene product, to this picture. Our results are compatible with models in which each of the three proteins interacts specifically with the other two or models in which one of the three proteins interacts with the other two.

(b) Critical Levels. The *gro*P-λπ system could also reflect the need in the cell for the lambda *P* protein to be in a proper proportion with the *dna*B and *gro*PC gene products. An excess of *P* protein might lead to abortive replication of some sort. According to this hypothesis, *gro*P mutations lower the level of host replication functions so that a compensating decrease in the level of the *P* protein is necessary for lambda growth.

Also possible are models involving specific interactions between two components and a proper ratio between the complex and the third component, and models in which *gro*PC is a regulatory element.

It would be interesting to look for specific interactions among these gene products, as a way of deciding between hypotheses. The elegant approach of Ratner (1974), involving covalent attachment of a protein to an agarose column for affinity chromatography could be used since the *dna*B protein has been purified (Shekman et al., 1974; Wickner and Hurwitz, 1974).

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