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Genetics

A bacterial mutation blocking P2 phage late gene expression

(*gro* mutant)

MELVIN G. SUNSHINE AND BRIAN SAUER

Department of Microbiology, University of Iowa, Iowa City, Ia. 52240; and Molecular Biology Department, University of California, Berkeley, Calif. 94720

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ABSTRACT **A mutant of *Escherichia coli* strain C has been isolated, called gro_{109}, that blocks bacteriophage P2 propagation by interfering with late gene expression. DNA replication proceeds normally in $P2^+$-infected gro_{109} cells, but late phage proteins are not made. Early P2 mRNA is made in normal amounts, but very little late mRNA can be detected. P2 mutants (P2 *ogr*) able to overcome the gro_{109} block have been isolated in which synthesis of late P2 mRNA and phage proteins is restored.**

The gro_{109} mutation is closely linked to the cluster of ribosomal genes at 64 min and is recessive to the wild-type (gro^+) allele. A P2 *ogr* mutation has been mapped on the left arm of the P2 genome, between the right-most known late gene (D) and the phage attachment site. P2 *ogr* can complement $P2^+$ in gro_{109} cells, indicating that *ogr* codes for a diffusible product.

Several aspects of the host cell's role in the development of the temperate bacteriophage P2 are demonstrated by the requirements for various *Escherichia coli* genes: *rep* (1), *dnaB* (Sunshine, unpublished data), *dnaE* (pol_{III}), and *dnaG* (D. Bowden, personal communication). These host functions are known to affect P2 DNA replication.

The work described here was undertaken to determine whether other host functions are involved in the propagation of P2. We report the isolation and characterization of (*a*) a host mutation (gro_{109}) that blocks P2 late gene expression, and (*b*) a P2 mutant (P2 *ogr*) that can overcome the gro_{109} block.

MATERIALS AND METHODS

Media. LB broth (LB) and LB agar (LA) are described by Bertani (2). LB agar is supplemented with 2.5 mM $CaCl_2$ for assaying P2. LB broth is supplemented with 80 mM $MgSO_4$ for preparation of Hy5 phage (3). TPG-CAA is described by Lindqvist (4). TPG complete medium contains 0.001% thymidine plus 5 μg/ml of each amino acid instead of casein amino acids. TPG low Met medium contains 19 amino acids at 5 μg/ml plus methionine at 0.25 μg/ml.

Growth and Purification of Phage. P2 phage stocks for isolating P2 DNA and mRNA were prepared and purified as described by Geisselsoder *et al.* (5). Hy5 was prepared as described by C. Bradley, P. L. Ong, and J. B. Egan (submitted for publication). Hy5 phage purification was the same as for P2.

Bacterial Strains. The bacterial strains used are described in Table 1. Strains designated C- and HF4704 were derivatives of *E. coli* strain C (6). All others are *E. coli* K derivatives.

Isolation of *E. coli* gro_{109}. Strain C-520 was UV-treated to 10% survival and allowed to grow through several divisions. About 10^5 cells were plated along with about 10^7 P2 vir_1. Surviving colonies were re-streaked and retested for ability to adsorb or release P2, and *rep* mutant colonies were eliminated by plating φX174 (15). One clone, number 109, did not release P2 phage, was killed by P2 vir_1 infection, was not *rep*, and was named C-2111.

Isolation of P2 Mutants Able to Form Plaques on Lawns of gro_{109} Cells. P2 mutants able to form plaques on a lawn of C-2111 were isolated by plating approximately 1 to 2×10^{10} phage on LA plates heavily seeded with C-2111 and incubating at 30°. Generally, one to two plaques appeared on such plates (frequency = 10^{-10}). Plaques were picked and reisolated through two passages on C-2111. Such P2 mutants are designated as P2 *ogr* for *o*ver *g*row on gro_{109}. The first P2 *ogr* mutants were isolated from stocks of P2 *lg*, which produces larger burst sizes than $P2^+$ (16). Subsequently, *ogr* mutants have been isolated from $P2^+$ stocks and shown not to differ from P2 *lg ogr* mutants other than in burst size. P2 *lg* ogr_1 will be referred to throughout this paper as P2 ogr_1.

Phage Strains. P2 vir_1 and P2 vir_3 are described by Bertani (17). Hy5 carries the late genes of P2 and the nonhomologous early region of phage 186 (18, 19). P2 *hy*dis* is a P2-related, heteroimmune hybrid phage (20). P2 conditional lethal mutants are described in the literature as follows: P2 tsB_{40} (21); P2 amA_{129} and P2 amB_{116} (22); P2 vir_{24} tsB_{40}, P2 vir_3 tsD_4, P2 vir_1 amD_6, and P2 vir_1 amT_5 (23); P2 vir_1 amU_{25}, P2 vir_1 amE_{30}, and P2 vir_1 amQ_{34} (10). P2 *ogr* mutations were combined with P2 conditional lethal mutations as follows: P2 vir_3 tsD_4 was used to select P2 vir_3 tsD_4 ogr_5, and a cross with P2 tsB_{40} yielded P2 vir_3 ogr_5. P2 vir_1 amQ_{34} was used to select P2 vir_1 amQ_{34} ogr_8. This strain was crossed with P2 vir_{24} tsB_{40} to yield P2 vir_1 ogr_8. P2 *lg* ogr_1 was crossed with P2 *hy*dis* to yield P2 *hy*dis* ogr_1, which was used to lysogenize strain C-2111. This lysogenic strain (C-2115) was infected with P2 amB_{116} to yield a P2 amB_{116} ogr_1 recombinant.

P1 Transduction Experiments. Phage P1 lysates were prepared and transduction experiments were performed as described by Sunshine and Kelly (24) with the following modification. After the bacteria in the adsorption mixture were washed with 1% saline, diluted aliquots were plated on LA plates and incubated for 4 hr at 37° before streptomycin (250 μg/ml) or spectinomycin (150 μg/ml) was added in soft agar overlays. This allowed the Str-s or Spc-s ribosomes to segregate before the antibiotic was added.

Phage Crosses. Phage crosses were performed as described by Lindahl (21) using either *E. coli* C-1a or C-520.

P2 DNA Synthesis. The extent of P2 DNA synthesis was determined by measuring incorporation of [^{3}H]thymidine into trichloroacetic acid-insoluble material in *uvrA* cells that had been treated with mitomycin C and infected with P2 according to the method of Lindqvist and Six (25).

Detection of P2 Phage Proteins. Labeled extracts of infected cells were prepared as described by Lengyel *et al.*

Abbreviations: *gro*, a bacterial mutation that blocks phage growth at a step subsequent to adsorption; UV, ultraviolet light; Ery, erythromycin; Spc, spectinomycin; Str, streptomycin.

Table 1. Bacterial strains

Collection no.	Relevant genotype	Origin/reference
C-1a	F^-, prototrophic	7
C-2	F^+	8
C-417	F^- *trp arg str met pur*	9
C-520	F^+ *supD*	10
C-1055	F^+, polyauxotrophic	9
C-2111	F^+ *supD* gro_{109}	C-520, see *text*
C-2115	F^+ *supD* gro_{109} (P2 *hy* dis* ogr_1)	C-2111 lysogenized with P2 *hy* dis* ogr_1
C-2116	F^+ *trp str* gro_{109}	Recombinant: C-2111 × C-417
C-2118	F^- *uvr thy str* gro_{109}	Transductant: C-2116 donor, HF4704 recipient
C-2119	F^- *ery*	From C-1a by mutation
C-2120	F^- *spc*	From C-1a by mutation
C-2121	F^- *str* gro_{109}	Transductant: C-2116 donor, C-1a recipient
C-2122	F^- *spc str* gro_{109}	From C-2121 by mutation
C-2123	F^- *ery str* gro_{109}	From C-2121 by mutation
C-2128	F^- *uvrA thy str*	From HF4704 by mutation
HF4704	F^- *uvrA thy*	11
KLF41/JC1553	F'141 gro^+	12
1100	F^- *end*	13
PM14	F^- *end* (Hy5)	1100 lysogenized with Hy5

The nomenclature used follows Taylor and Trotter (14).

(26), with the following modifications: TPG low Met medium was used in place of HF medium. Cells were UV-irradiated for 4½ min, instead of 3 min, to diminish host protein synthesis background. Infected cells were diluted into TPG complete low Met medium at zero time and then labeled with [^{35}S]methionine (2.6 μCi/ml, specific activity 1.5 Ci/mmol). The time for administration of the radioactive methionine pulse was selected after determining lysis time in the UV-treated cells. Lysis of both C-2128 infected with P2 vir_3 and C-2128 infected with P2 vir_3 ogr_5 occurs at 40 min after infection. Lysis of C-2118 infected with P2 vir_3 ogr_5 occurs at 60 min after infection. No lysis is observed for C-2118 infected with P2 vir_3. Labeling was from 5 to 35 min for gro^+ cells, and from 15 to 45 min for gro_{109} cells. Labeled extracts were analyzed as described (26) on 10% polyacrylamide/dodecyl sulfate gels.

Extraction and Hybridization Analysis of Phage mRNA. Ten-milliliter cultures of cells were grown and infected as described above for labeled protein extracts, except that the infected cells were not UV-irradiated. The cultures were pulse-labeled at the indicated times for 3 min with [^{3}H]uridine (10 μCi/ml; specific activity 28 Ci/mol). Cells were collected, lysed, and extracted with phenol as described (5). Extracts were then stored frozen at −20° for no longer than 5 days. DNA for hybridization was extracted from Hy5 and P2 vir_1 by the procedure of Thomas and Abelson (27). Hybridization conditions were as described (5) using 2–4 μg of denatured DNA per filter. Hybridization to Hy5 DNA gives a direct measure of late P2 mRNA, since the early region of this phage is not homologous with P2 (19).

RESULTS

Growth of phages in gro_{109} strains

A bacterial mutant that blocks the growth of phage P2 was isolated from *E. coli* C after mutagenesis with UV. The burst size of P2 on this mutant strain (*E. coli* gro_{109}) is less than 0.1 phage per cell, as compared to 160 phage per cell on the parental strain. P2$^+$ plates on *E. coli* gro_{109} at an efficiency of 10^{-10}. The small plaques formed at this frequency represent P2 *ogr* mutants (*o*vercoming the *gro* block), which grow well on both gro^+ and gro_{109} *E. coli*. None of the nine independently isolated *ogr* mutants is *am* or *ts*, as shown by normal burst sizes and efficiencies of plating when grown on *E. coli* sup^- at 30° and 42°.

The gro_{109} block appears to be specific for P2 and the P2-related phages P2 *hy*dis*, 299, and Wϕ. Phages P1, ϕX-174, R17, Qβ, λ, and T7 all plate normally on gro_{109} lawns.

Mapping of gro_{109} in *E. coli*

To find the chromosomal location of the gro_{109} mutation, $F^+ \times F^-$ crosses were performed, and linkage between gro_{109} and *str* was detected. This linkage was further demonstrated by P1 transduction from a str^r gro_{109} strain (C-2116) into a str^s strain (C-2), selecting for Str-R. Of 100 transductants tested, 58% simultaneously obtained the ability to block the growth of P2.

Nomura and Engbaek (28) have presented data indicating that the cluster of ribosomal protein genes near the *str* locus is transcribed as a single unit, a "ribosomal protein operon," and that the direction of transcription is in the order of *ery, spc, str,* and *fus*. These markers were used to more definitively map the location of gro_{109}. Various ery^r, spc^r, and str^r derivatives of C-2116 and C-1a were constructed, and P1 lysates were prepared on them. The data in Table 2 show the results of two transduction experiments. In experiment A, Str-R was selected, and linked markers from the donor strain were efficiently transferred: 78% (71/91) of the transductants were spc^s, 77% (70/91) were ery^r, and 74% (67/91) were gro_{109}. These cotransfer frequencies, along with analysis of the various classes of transductants, indicate the order gro_{109}, *ery, spc, str*. The data presented in experiment B are consistent with this order. One experiment, not listed in

Table 2. Mapping of gro_{109} by P1 transduction

Transduction	Selection	Genotypes of transductants	Frequency
A. Donor: C-2123 (*gro*$_{109}$ *ery*^{r} *spc*^{s} *str*^{r})	Str-R	*gro*$_{109}$ *ery*^{r} *spc*^{s}	66/91 (73%)
Recipient: C-2120 (*gro*$^+$ *ery*^{s} *spc*^{r} *str*^{s})		*gro*$^+$ *ery*^{s} *spc*^{r}	20/91 (22%)
		gro$^+$ *ery*^{r} *spc*^{s}	4/91 (4%)
		gro$_{109}$ *ery*^{s} *spc*^{s}	1/91 (1%)
B. Donor: C-2122 (*gro*$_{109}$ *ery*^{s} *spc*^{r} *str*^{r})	Spc-R	*gro*$_{109}$ *ery*^{s} *str*^{s}	37/96 (38.5%)
Recipient: C-2119 (*gro*$^+$ *ery*^{r} *spc*^{s} *str*^{s})		*gro*$_{109}$ *ery*^{s} *str*^{r}	36/96 (37.5%)
		gro$^+$*ery*^{r} *str*^{s}	9/96 (9.4%)
		gro$^+$ *ery*^{s} *str*^{s}	8/96 (8.3%)
		gro$^+$ *ery*^{s} *str*^{r}	3/96 (3.1%)
		gro$_{109}$ *ery*^{r} *str*^{s}	3/96 (3.1%)
Deduced order: *gro*$_{109}$ - *ery* - *spc* - - *str*			

Transductions were performed as described in *Materials and Methods*.

Table 2, showed 90% (34/38) cotransduction of gro_{109} with *ery*^{r}.

Dominance of *gro*$^+$

The close linkage of gro_{109} to *str* allowed us to determine whether gro_{109} is dominant or recessive by construction of diploid strains. The episome, F'141, carries genetic material of *E. coli* covering *str* and adjacent genes (12). F'141-containing derivatives of C-2116 were prepared by allowing KLF41/JC1553 (F' *gro*$^+$) to mate with C-2121 (F$^-$ *str*^{r} *gro*$_{109}$) for 40 min and plating on LA for colonies. Twenty five colonies were reisolated and tested for maleness by ability to plate phage R17. Five strains were male by this test, and all five plated P2$^+$ with normal efficiency. All five diploid strains were tested for ability to be cured by acridine orange (1). After acridine treatment, all five strains yielded F$^-$ gro_{109} clones at high frequency. This indicates that *gro*$^+$ is dominant to gro_{109} and that the *gro* gene produces a product that allows P2 development.

P2 DNA synthesis

P2 DNA synthesis in gro_{109} cells was measured by the cumulative incorporation of [^{3}H]thymidine into acid-insoluble material. Fig. 1 shows the incorporation of label after P2$^+$ and P2 ogr_1 infection of gro_{109} strain C-2118. The rate and level of incorporation were identical for both phage strains. Thus, P2$^+$ DNA replicates as well as does P2 ogr_1 DNA in gro_{109} *E. coli*.

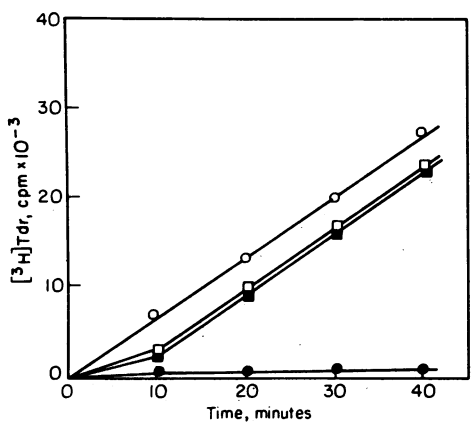


FIG. 1. P2 DNA synthesis in C-2118 *uvrA thy* gro_{109}. Cells were pretreated with 60 μg/ml of mitomycin C for 10 min to selectively suppress host DNA synthesis, washed, resuspended in TPG -CAA plus 2 μg/ml of unlabeled thymidine, infected, labeled with [^{3}H]thymidine (50 μCi/ml, specific activity 5 Ci/mmol), and sampled at the given times. O, Untreated cells; ●, mitomycin pretreated cells; □, mitomycin pretreated cells plus P2 *ogr*$^+$; ■, mitomycin pretreated cells plus P2 ogr_1.

Synthesis of phage proteins

Electrophoresis of ^{35}S-labeled proteins from infected and uninfected *gro*$^+$ and gro_{109} strains shows that late phage proteins are not present in P2$^+$-infected gro_{109} cells, although the early protein B can be seen (Fig. 2). The P2 ogr_5 mutant, on the other hand, can synthesize phage late proteins in gro_{109} cells. Since no host protein migrates to the same position as P2 late protein H, we measured synthesis of this protein from tracings by a microdensitometer. The gro_{109} mutation reduced H protein synthesis by P2 *ogr*$^+$ at least 40-fold when compared to P2 ogr_5, to a level indistinguishable from that for uninfected cells.

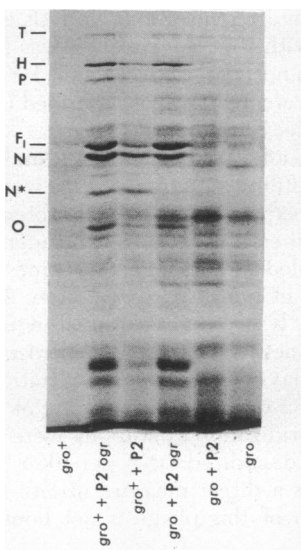


FIG. 2. Pattern of protein synthesis in uninfected and infected *gro*$^+$ and gro_{109} cells. C-2128 (*gro*$^+$) and C-2118 (gro_{109}) were UV-irradiated, infected, and labeled with [^{35}S]methionine as described in *Materials and Methods*. Infection was with P2 vir_3 and P2 vir_3 ogr_5, as indicated. Identification of P2 proteins is described by Lengyel *et al.* (26, 29) and Lengyel and Calendar (30). Proteins H, P, F_I, N, N*, O, and F_{II} are P2 late proteins; B is an early protein.

Table 3. Detection of P2 mRNA

Bacteria	Phage	mRNA (% input hybridized)* Early 5 min	Late 20 min	Late 45 min
gro^+	—	0.018	0.003	—
gro^+	P2	0.46	1.5	—
gro^+	P2 ogr_5	0.38	0.53	—
gro_{109}	—	0.002	0.003	0.003
gro_{109}	P2	0.43	0.039	0.20
gro_{109}	P2 ogr_5	0.21	1.0	3.6

Cultures were pulse-labeled for 3 min at the indicated times after dilution into warm medium, as described in *Materials and Methods*. Values are the average of two or three determinations. Counts retained on blank filters averaged 80 cpm (range: 30–150 cpm) and were subtracted from values obtained on DNA-loaded filters. Both P2 phage strains carried the vir_3 marker.

* Input: 1.5 to 2.2 × 10^5 cpm, early; 3 to 8 × 10^5 cpm, late.

Synthesis of early P2 mRNA

No detectable late P2 mRNA is made early after infection (5). Hence, presence of early P2 mRNA may be assayed directly by hybridization to P2 DNA. Table 3 shows the results of hybridization experiments in which gro^+ and gro_{109} cells were infected with $P2^+$ and then labeled with [^{3}H]uridine, followed by extraction of RNA and hybridization to DNA from the appropriate phage. At 5 min after infection with P2 vir_3, approximately equal amounts of early P2 mRNA are present in both gro^+ and gro_{109} hosts. Thus, gro_{109} does not appear to affect early P2 transcription.

Synthesis of late P2 mRNA

The amount of late P2 mRNA made in P2-infected *E. coli* gro_{109} was measured by hybridization to phage Hy5, which carries only late P2 genes and is not homologous to P2 in the early region (19) (Table 3). Very little late P2 mRNA was detected in P2 ogr^+-infected gro_{109} cells at 20 min, about 2% of that found in P2 ogr^+-infected gro^+ cells. At 45 min, which is after the normal lysis time, somewhat more late P2 mRNA was detected in P2 ogr^+-infected gro_{109} cells. Late P2 mRNA is synthesized at normal levels in P2 ogr_5-infected *E. coli* gro_{109} or gro^+ cells. Thus, the gro_{109} mutation greatly reduces late P2 transcription.

Dominance of P2 *ogr* mutants

To determine whether the *ogr* mutation affects a site or a phage gene product, complementation tests were performed. The results of one such test, shown in Table 4, demonstrate that P2 tsD_4 ogr_5 can complement P2 ogr^+ in *E. coli* gro_{109} at 42° to produce a burst of both parental phage types. The P2 *ogr* mutant thus allows expression of the late gene D on the P2 ogr^+ genome. We conclude that the *ogr* mutation is *trans*-dominant to the ogr^+ allele and that P2 *ogr* mutants supply a gene product which allows phage development in gro_{109} cells.

Mapping of a P2 *ogr* mutation

The phage crosses listed in Table 5 place the *ogr* gene on the left arm of the P2 genome between the right-most tail gene D and the phage attachment site. Crosses 1 and 2 place the *ogr* gene to the left of the attachment site, as Lindahl (31) has shown that for markers which span the P2 attachment region (*att*), recombination is largely *int* promoted (approximately 100-fold greater than non-*int* promoted recombination). Cross 3 indicates that ogr_5 is located closer to gene D than to gene E. Crosses 4 and 5 place *ogr* between gene D and the phage attachment site.

Table 4. Complementation between P2 *ogr* and P2 ogr^+ in gro_{109} cells

Host strain and relevant genotype	Infecting phage	Phage burst
C-2111	P2 vir_3	0.04
gro_{109}	P2 vir_3 tsD_4 ogr_5	0.01
	P2 vir_3 ogr_5	173
	P2 vir_3 + P2 vir_3 tsD_4 ogr_5 mixed	54 (16 P2 vir_3 + 38 P2 vir_3 tsD_4 ogr_5)

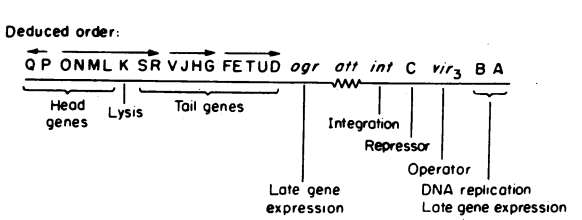
Complementation tests were performed at 42° as described by Lindahl (21).

DISCUSSION

We have shown that an *E. coli* mutation, called gro_{109}, blocks the multiplication of bacteriophage P2 and prevents

Table 5. Phage crosses

Phage cross	Parental types	Selection	Frequency of unselected marker
1	*tsD ogr* + / + + *tsB*	ts^+	224/250 = ogr^+
2	+ *ogr* *amB* / *amD* + +	am^+	3/99 = ogr^+
3	+ *tsD* *ogr* / *amE* + +	am^+ts^+	63/75 = ogr^+
4	+ *tsD* *ogr* / *amU* + +	ts^+ogr	73/97 = *amU*
5	+ *tsD* *ogr* / *amT* + +	ts^+ogr	69/100 = *amT*



The parental phage strains are described in detail in *Materials and Methods*. The gene order deduced is inserted into a comprehensive genetic map, derived from Lindahl (23) and Sunshine *et al.* (10). Arrows indicate direction of transcription. Phage were treated with UV to 50% survival before crossing.

the transcription of late genes. Mutants of P2 that overcome the gro_{109} block have been isolated and are called P2 *ogr*. The *trans*-dominance of P2 *ogr* in mixed infection with P2 ogr^+ indicates that P2 *ogr* codes for a diffusible gene product.

The gro_{109} mutation is recessive to the wild-type allele, and is tightly linked to a cluster of ribosomal protein genes near the *str* locus. We suspect that gro_{109} may be a ribosome-associated protein. This view is strengthened by the finding of Jaskunas and Nomura that a gro^+ gene lies within the cluster of ribosomal protein genes from the *str-spc* region of *E. coli* K-12 that are carried by λ *spc*1 and λ *spc*2 (ref. 32; personal communication).

Replication of P2 ogr^+ DNA, which requires the P2 early gene products A and B (4, 22), proceeds normally in gro_{109} cells. P2 late proteins are not made, however, and the production of P2 late mRNA is greatly reduced. The production of early mRNA and early protein B appears unaffected.

The expression of P2 late genes may thus involve an interaction between a phage gene product (ogr^+) and a component of the translation apparatus (gro^+). Such an interaction could occur in one of several ways. For example, transcription of late P2 genes may require a host protein factor which is also needed for translation. This would be analogous to the situation found in the production of Qβ phage RNA (33, 34). Alternatively, P2 late gene transcription may be greatly influenced by the translational process itself. Stent (35, 36) has suggested that ribosome binding to nascent mRNA could enhance the rate of transcription. Since P2 late mRNA but not early mRNA synthesis is affected by gro_{109}, a third alternative could be that the P2 ogr^+ product somehow modifies the *E. coli* $gro_{109}{}^+$ product, allowing transcription of P2 late genes. The gro_{109} and *ogr* gene products must be purified before we can distinguish between the above possibilities.

It is clear that the P2 *ogr* gene is essential for phage production in *E. coli* gro_{109}. Although conditional lethal mutants of P2 ogr^+ have not yet been isolated, we suspect that ogr^+ is an essential gene in *E. coli* gro^+, by analogy with the related phage 186. The essential gene of phage 186, defined by am_{17}, maps at a location corresponding to *ogr* and is needed for cell lysis and phage particle formation (S. Hocking and J. B. Egan, personal communication).

The unusual location of *ogr* on the P2 genetic map raises the question of how its expression might be controlled. Sunshine (unpublished results) has shown that *ogr* is not under direct immunity control nor under split operon control as is the *int* gene (37). The *cis*-acting product of P2 gene A is needed for *ogr* gene expression (Sunshine, unpublished results). In this respect, the *ogr* gene is similar to the 18 P2 late genes, and unlike the early gene B (23, 37). It seems unlikely that *ogr* is part of the $\overrightarrow{\text{FETUD}}$ transcription unit, since the *ogr* product is needed for expression of those genes.

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