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Bacterial *rep*⁻ Mutations That Block Development of Small DNA Bacteriophages Late in Infection

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Several related mutants of *Escherichia coli* C have been isolated that block the growth of the small icosahedral DNA phages ϕX174 and S13 late in infection. Phage G6 is also blocked, at a stage not yet known. Growth of the filamentous phage M13, though not blocked, is affected in these strains. These host mutations co-transduce with *ilv* at high frequency, as do *rep*⁻ mutations. However, the new mutants, designated *gro*L⁻, differ from previously studied *rep*⁻ mutants in that they permit synthesis of progeny replicative-form DNA. The *gro*L⁻ mutants are blocked in synthesis of stable single-stranded DNA of ϕX174 and related phages. They are *gro*⁺ for P2. Evidence that *gro*L⁻ mutations and *rep*⁻ mutations are in the same gene is presented. Spontaneous mutants (*ogr*) of ϕX174, S13, and the G phages can grow on *gro*L⁻ strains. The *ogr* mutations are located in the phage's major capsid gene, F, as determined by complementation tests. There are numerous sites for mutation to *ogr*. Some mutations in genes A and F interfere with the *ogr* property when combined with an *ogr* mutation on the same genome. The *ogr* mutations are *cis* acting in a *gro*L⁻ cell; i.e., an *ogr* mutant gives very poor rescue of a non-*ogr* mutant. The wild-type form of each G phage appears to be naturally in the *ogr* mutant state for one or more *gro*L⁻ strains. It is suggested that a complex between F and *rep* proteins is involved in phage maturation. The A protein appears to interact with this complex.

It is known that T4, T7, λ, P2, and other phages require for their development certain host proteins that are not essential to the viability of the cell (2, 3, 10–12, 15, 32, 33, 40). The requirement of a phage for a given host protein was revealed when a mutation in the host blocked phage development. In many of the above cases a mutation in a phage gene can overcome the block produced by the host mutation; in some of these latter cases there is evidence that a phage-host protein complex is formed in a "lock and key" fashion. A classic example of a "lock and key" interaction are host mutants resistant to phage adsorption and mutant phages that overcome this host block (24).

For the small DNA icosahedral phages (ϕX174, S13, the G phages) and the filamentous phages (M13, f1, fd), one protein, non-essential for the host, has been shown to be needed, the product of the *rep* gene (5). The protein product of *rep* is required for progeny replicative-form (RF) DNA synthesis in these phages but not for parental RF synthesis (5). Phage development in *rep*⁻ mutants is blocked at a point at which the parental RF DNA has formed a partial single strand (8, 9). Although *rep*⁻ host mutants are viable, they have certain abnormalities in host DNA replication. In the *rep*⁻ mutant the growing forks of the host DNA move at a slower than normal rate (20, 21). No phage mutants have been found that overcome the *rep*⁻ block, until the present work. Phage P2 also requires the *rep* protein for progeny RF synthesis (1).

We describe here a new class of non-essential host mutants, which while ϕX *gro*⁻ are nevertheless P2 *gro*⁺. These mutants are designated *gro*L⁻ (late block). In *gro*L⁻ hosts, progeny RF synthesis of ϕX174-related phages is normal, but development is blocked at a late stage, just before or during the synthesis and encapsidation of single-stranded (SS) DNA. Phage mutants (*ogr*) can overcome the *gro*L⁻ block, and their properties are described here. The filamentous phage M13 grows more poorly on one of the *gro*L⁻ strains than on *gro*⁺, although the stage at which M13 growth is affected is not yet known. *gro*L⁻ mutations map very close to *rep* on the *E. coli* chromosome, and from other evidence presented here they appear to be a new type of mutation of the *rep* gene. (All the strains that permit phage adsorption but yield no phage are designated *gro*⁻. The minus sign, though not in strict accordance with Demerec's terminology, is permitted for emphasis [26, p. 7] and is necessary here to

make the text more readable. When the gro^- or rep^- strain is given a number, the minus sign is omitted, e.g., *gro*89. Any strain that is found to be blocked in phage progeny RF synthesis is given the designation *rep* but will be referred to here either as rep^- or gro^- according to the context. The term *gro*L^- designates those gro^- strains that permit normal amounts of synthesis of progeny RF in 30 μg of chloramphenicol [CM] per ml and normal or near-normal amounts of progeny RF in the absence of CM.)

A class of mutants that are ϕX gro^- and P2 gro^+ was isolated by M. Iwaya (Ph.D. thesis, Harvard University, Cambridge, Mass., 1971) and was designated *repB*. Mutants of this class form small amounts of progeny RF and appear to produce defective particles of low density.

MATERIALS AND METHODS

Bacterial and phage strains. The parental strain for the new gro^- mutants was *E. coli* C1a, obtained from E. Six. *E. coli* rep_3, isolated by Denhardt et al. (5), was obtained from D. Ray. *E. coli* C-1415 is a strain into which rep_3 was transduced by Calendar et al. (1) and was obtained from R. Calendar. The recipient strain for this transduction, C-1412 (ilv^- met^- his^-), was also obtained from R. Calendar. Of the gro^- strains studied here, *gro*40, *gro*85, *gro*87, and *gro*89 are transductants of gro^- into C-1412. Strain *gro*121 is not a transductant.

Strain AB1206 carrying the F'14 episome was obtained from Barbara Bachman. The suppressing strain C-520, isolated by M. Sunshine, was obtained from E. Six. M. Levinthal supplied the transducing phage P1clr100CM, isolated by Rosner (27). Phage λCI857 was obtained from R. Somerville. The G phages were obtained from G. N. Godson, ϕX *am*D56 from Marie Hayashi, and P2 vir_2 from M. Sunshine.

M9 medium was prepared according to the formula of Miller (26), except that $FeCl_3$ was added to 10^{-5} M and Casamino Acids were added to 0.05%. For phage adsorption in M9 cultures, $MgSO_4$ was added to 2×10^{-2} M. For M9 plates, Casamino Acids were omitted, and specific amino acids were added to 40 μg/ml.

Isolation of gro^- mutants. Log-phase, 0.5-ml broth cultures of *E. coli* C1a were mutagenized by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (670 μg/ml) for 15 min without shaking and then diluted 10^3 times into broth and allowed to grow for 3.5 h at 37°C to permit segregation of gro^- cells. About 2,000 surviving cells were plated together with 2×10^6 wild-type S13 particles on each plate, and the plates were incubated at 37°C. The numbers of cells and phage used and the temperature must be carefully controlled, since the gro^- cells would be killed if infected. There is only a narrow phage concentration range in which gro^- cells will survive. The optimum number of phage and cells was determined by tests with strain rep_3.

The selection depends on the formation of microcolonies of gro^- cells before they can be attacked by the phage released from gro^+ cells on the plate. Only a few of the cells of a gro^- microcolony are killed; the rest grow into a macrocolony and protect each other from further phage attack.

About 10 colonies grew up on each phage-seeded plate. Each colony was dipped into 1 ml of broth, streaked, and then repicked and grown up with shaking in a small tube and tested in batches of 48 by the chloroform method for ability to adsorb phage S13. Adsorption is good even when the culture has grown out of log phase. Out of 250 colonies picked, 24 were good adsorbers and gave no burst of S13. These strains were designated gro^-.

Transduction, scoring, and curing. Transduction was carried out by the methods of Rosner (27) and of Goldberg et al. (14). Each gro^- strain was lysogenized with P1clr100CM and a transducing lysate was made of each. It should be noted that this phage is destroyed by even brief vortexing with chloroform. The ilv^- recipient, *E. coli* C-1412, was transduced with each lysate, and ilv^+ colonies were picked and streaked on plates lacking isoleucine and valine. The ilv^+ transductants were grown up and scored for being S13 gro^+ or S13 gro^-.

The scoring for the gro^- property was complicated by the fact that the transduced cultures generally yield some P1-resistant cells in the course of growth even at 25°C and even when the broth is 5×10^{-3} M in sodium citrate. The P1-resistant cells are also S13 and ϕX resistant. Thus, a culture might appear to be gro^- when it is actually a resistant gro^+ culture, and a simple spot test on phage-seeded plates might give false results.

To score the gro^- or gro^+ property in cultures containing some resistant cells, a test based on reduction of efficiency of phage plating (EOP) was used. A mixture of the poorly adsorbing indicator strain, *Shigella dysenteriae* Y6R, and the well-adsorbing gro^- strain was plated with a phage S13 gene H adsorption mutant, *t*281. One drop of Y6R plus 10 drops of gro^- culture were used. In this plating test a gro^- strain gives a very big decrease in EOP of *t*281, whereas a gro^+ strain gives 100% EOP of the phage. This test can be used to distinguish gro^- from gro^+ strains of *E. coli* C even if 90% of a culture consists of resistant cells. In this way it was found that 22 out of the 24 gro^- strains were closely linked to *ilv*.

To study DNA synthesis and other properties of the gro^- strains, gro^- transductants were cured of P1 by overnight growth in broth at 40°C. Sensitivity to CM was determined by streaking on CM plates and indicates that the P1 phage has been lost. Single colonies were picked from the cured cultures, and then these were retested for adsorption. Only good adsorbers were used.

Complementation and rescue. Complementation tests were carried out at 41.5 and 42.0°C in *E. coli* C1a using the ϕX174 temperature-sensitive *ogr* mutants *ogr*89-1, *ogr*89-5, and *ogr*89-105. Cells grown in broth to 2×10^8/ml were infected at high temperature with each phage at a multiplicity of infection of 4 in the presence of 10^{-2} M $MgSO_4$. The infected cells were diluted 10^4 times into warm broth at 7 min, and at 60 min the growth tubes were chloroformed, di-

luted, and plated. Each growth tube was plated on strains *gro*89 and C1a for the *ogr* mutant burst and on strain C-520 for total burst.

Rescue experiments in gro^+ and *gro*89 cells were carried out as for the complementation tests except that the temperature was 36°C. Plating was on the mixed indicator (35), which distinguishes S13 amber from non-amber mutants.

Analysis of DNA. Infection was carried out as follows. Cells were grown in M9 medium to 2×10^8/ml. $MgSO_4$ was added to 2×10^{-2} M, and dialyzed phage was added to a multiplicity of infection of 5 to 10. When CM was used it was added 3 min before infection. A 200-μCi amount of [^{3}H]thymidine (Amersham/Searle; 15 Ci/mmol) was added to each 5-ml culture. The cells were chilled at the end of the pulse, centrifuged three times, and resuspended each time in 0.05 M borate-0.006 M EDTA. Gentle cell lysis and centrifugation of extracts in high-salt sucrose gradients was carried out as described by Francke and Ray (8), except that in later experiments a 15-min heating period at 60°C preceded Pronase treatment to improve release of SS and in those cases Pronase treatment was for 30 min at 37°C. Centrifugation was in the SW27 rotor of the Beckman ultracentrifuge at 5°C, and centrifugation times varied from 16 to 20 h at 25,000 rpm in different experiments. One-milliliter fractions were collected from the top of the tube. Samples (0.02 ml) were added to 5 ml of a scintillation fluid composed of 80 parts water, 100 ml of Aquasol, and 0.5 part glacial acetic acid.

RESULTS

***gro*$^-$ strains.** The gro^- colonies surviving on phage-seeded plates were distinguished from resistant colonies and from parental gro^+ colonies by the fact that they adsorbed phage as well as the parental strain but yielded about one S13 phage particle per cell or less. Ten percent of the colonies tested were gro^- (24 colonies). The frequency of mutation to gro^- was about 5×10^{-4}. The gro^- strains were then tested for co-transduction frequency with *ilv*, because we wished to obtain gro^- strains that were different from rep^- and it has been shown that rep_3 co-transduces with *ilv* at high frequency (1). We were seeking gro^- strains that did not co-transduce with *ilv*. Therefore, each of the 24 gro^- strains was used as a donor for P1 transduction, and about 12 ilv^+ transductants from each transduction were scored for phage growth by the EOP reduction method (see Materials and Methods). Twenty-two of the mutant strains showed high co-transduction frequencies with *ilv*. Thus, it appeared possible that these 22 gro^- mutants might all be rep^- strains. However, nine of these gro^- strains were gro^+ for P2, which is not a rep^- characteristic. Furthermore, most of these P2 gro^+ strains, when plated with high concentrations of ϕX174 or G4, yielded phage mutants, designated *ogr*, and phage mutants do not arise on rep^- strains (4). Several strains that were gro^+ for P2 were studied for DNA synthesis to see if, like rep^-, they were blocked in progeny RF synthesis (5).

DNA synthesis in strains that are ϕX gro^- and P2 gro^+. The five phenotypically different gro^- strains that were examined fell into two different groups on the basis of their stage of block of ϕX DNA synthesis. The results given below are summarized in Table 1. All the strains were examined first for their ability to form progeny RF in CM (30 μg/ml). Both ϕX174 and S13 accumulate progeny RF when a wild-type host is infected in 30 μg of CM per ml (31, 35). If the CM dose is increased to 100 μg/ml, only parental RF is formed (35). No SS is formed in 30 μg of CM per ml. It is convenient to use CM (30 μg/ml) when measuring progeny RF synthesis, because the amount of RF that accumulates is much greater than in infection without CM. Of the five strains analyzed, two showed a normal amount of progeny RF synthesis in CM, two showed no progeny RF synthesis, and one strain was leaky. The strains that formed progeny RF with CM also formed progeny RF in the absence of CM. All five strains failed to form SS.

The first two gro^- strains that were examined were the ones that yielded normal amounts of progeny RF. These two strains (*gro*87 and *gro*89) were studied first because of their striking ability to yield phage *ogr* mutants (see below). In the first experiment, these two gro^- strains were compared with the isogenic gro^+ and rep_3 strains by labeling with [^{3}H]thymidine at a late time (25 to 45 min) after infection with ϕX174 in 30 μg of CM per ml. Figure 1 shows that the two gro^- strains form approximately the same amount of labeled progeny RF as gro^+, whereas rep_3 forms no

TABLE 1. *DNA synthesis by bacterial mutants that are ϕX gro^- and P2 gro^+*

Host mutant	Parental RF	Progeny RF: No CM	Progeny RF: 30 μg of CM per ml	Yields phage *ogr* mutants
*gro*85	+	+	+	+
*gro*87	+	++++[a]	++++	+
*gro*89	+	++	++++	+
*rep*40	+	0	0	+
*rep*121	+	0	0	0
gro^+	+	++++	++++	–[b]

[a] Four plus signs denote the maximum amount of progeny RF, i.e., the amount formed in the gro^+ strain.

[b] Only gro^- strains yield *ogr* mutants.

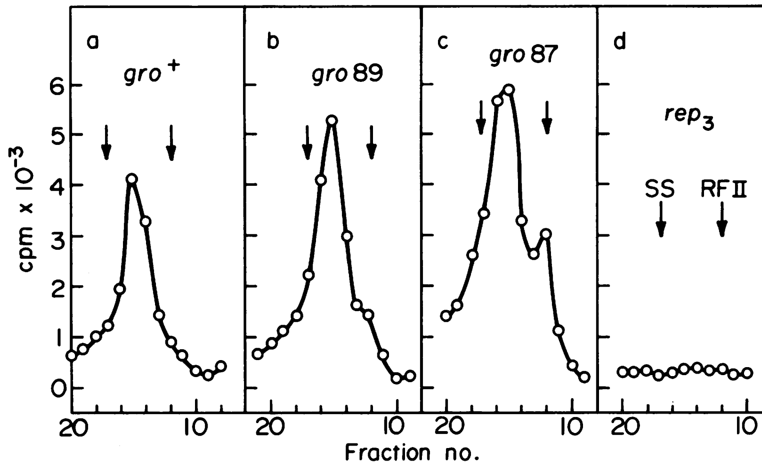

FIG. 1. *Synthesis of late RF in CM (30 μg/ml) after infection of the four isogenic strains gro^+, gro89, gro87, and rep_3 with wild-type φX174 phage. The cells were grown to 2×10^8/ml in M9 medium, and $MgSO_4$ was added to 2×10^{-2} M. CM was added 3 min before infection at a multiplicity of infection of 10 at 37°C, and 200 μCi of [³H]thymidine was added to a 5-ml infected culture 25 min after infection. The cultures were chilled at 45 min after infection, and then gently lysed by the procedure of Francke and Ray (8) and sedimented in a 5 to 20% high-salt neutral sucrose gradient (8). Sedimentation was from right to left; the arrows mark the position of SS and RFII.*

progeny RF. The original rep_3 strain (5) also formed no progeny RF (data not shown). Results with S13 were the same as for φX174, as were the results when the labeling period was from 15 to 35 min postinfection.

CM was omitted to determine if the two gro^- strains permit the formation of stable SS. No SS is observed even by 40 min (Fig. 2 and 3). We conclude that these two gro^- strains (*gro*87 and *gro*89), though forming normal amounts of progeny RF, are blocked in formation of stable SS. (In infection with φX-related phages, a block in SS formation usually cannot be distinguished from a block in encapsidation.)

These two strains that form progeny RF are designated *gro*L^-.

A third isogenic gro^- strain, *gro*40, formed almost no RF at late times in the presence of CM, in contrast to the substantial amount of RF formed by the gro^+ strain (Fig. 4). Since *gro*40 forms no progeny RF, it is classified as a rep^- strain. However, this rep^- strain is remarkable in that it gives rise to phage *ogr* mutants when plated with high concentrations of phage. It will be shown below that the fact that *gro*40 yields *ogr* mutants is the main evidence that rep^- and *gro*L^- mutations are in the same gene. Strain *gro*40 will be called *rep*40 in certain contexts here.

A fourth strain, *gro*85, shows much less RF synthesis in CM than strains *gro*87 and *gro*89 (Fig. 5), although it shows more than rep_3 or

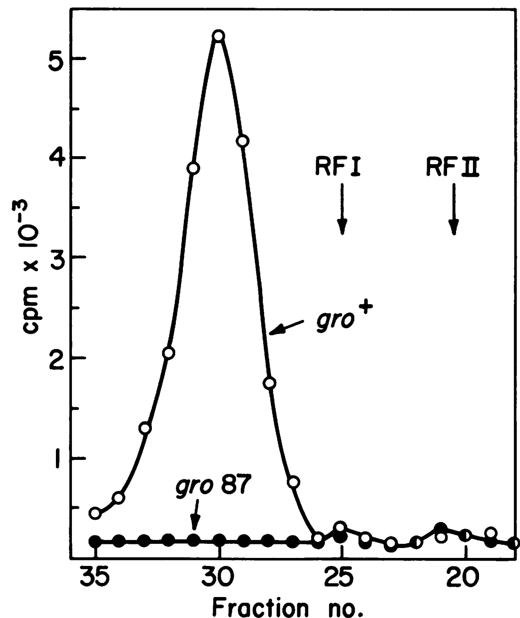

FIG. 2. *Absence of SS synthesis in strain gro87 as compared to presence of SS in the gro^+ strain. The gro^+ and gro87 cultures were infected with the φX174 lysis mutant amE3 at a multiplicity of infection of 7 at 37°C in M9 medium. [³H]thymidine (200 μCi) was present from −1 min to 40 min after infection, when the 5-ml cultures were chilled. Gentle lysis and sedimentation were as described in Materials and Methods.*

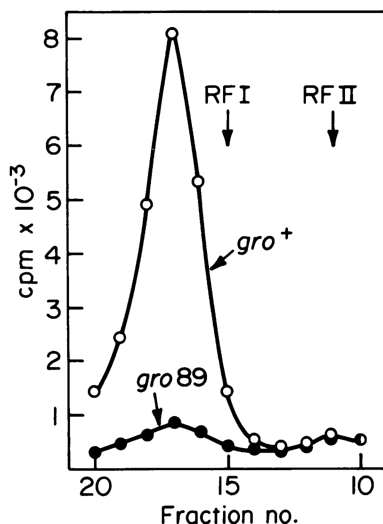

FIG. 3. *Absence of SS synthesis in strain gro89 as compared to presence of SS in gro$^+$. Procedures were as for Fig. 2.*

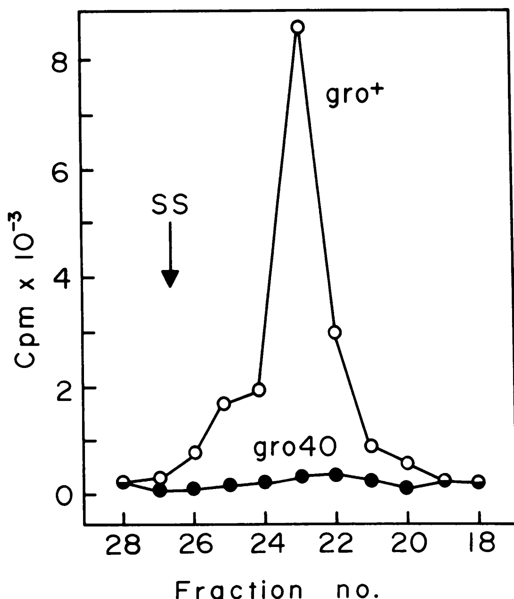

FIG. 4. *Comparison of formation of late RF in CM (30 μg/ml) in strains gro40 and gro$^+$ after infection of φX amE3. Procedures were as for Fig. 1.*

*gro*40 (*rep*40). It appears to be a leaky *rep*$^-$ strain. It was studied further because it yields several phage *ogr* mutants. From its *ogr* mutant-plating properties, strain *gro*85 has proved to be a link between the *gro*L$^-$ strains and the non-leaky strain *rep*40.

All these strains except *rep*40 were examined for progeny RF synthesis in the absence of CM at fairly early times after infection, when progeny RF synthesis is most abundant. Cultures were infected with φX *am*E3 in M9 medium at 37°C and were labeled with [^{3}H]thymidine from 10 to 18 min after infection. By this time parental RF synthesis is over, as seen by the curve for rep_3 in Fig. 6. It is also seen in Fig. 6 that the two *gro*L$^-$ strains, *gro*87 and *gro*89, both form progeny RF in the absence of CM, but *gro*87 forms about the same amount as the *gro*$^+$ strain whereas *gro*89 forms about half as much. This result is in contrast to the equal ability of *gro*87 and *gro*89 to form progeny RF in CM. It is also seen that both *gro*87 and *gro*89 show small peaks in the SS region. This DNA has not yet been analyzed.

Strain *gro*85 forms only a small amount of progeny RF in the absence of CM (Fig. 6). This result serves to support its classification as a leaky *rep*$^-$ strain.

DNA synthesis was also studied for another φX *gro*$^-$ strain that is P2 *gro*$^+$ but, unlike the four strains discussed above, is not an *ogr* mutant yielder. This strain, *gro*121, forms parental RF but, like rep_3, forms no progeny RF in CM (Fig. 7). Strain *gro*121 will be called *rep*121 in certain contexts here.

Measurement of leakiness. All the *gro*$^-$ strains were tested for burst size of wild-type S13 early in this work and were found to yield on the order of one particle per cell. However, when progeny RF synthesis is measured, labeling is done rather late in infection, beginning at 15 or 25 min postinfection and extending beyond the normal lysis time. If a *gro*$^-$ strain were able to give rise to a substantial burst of phage particles when lysis was delayed, the

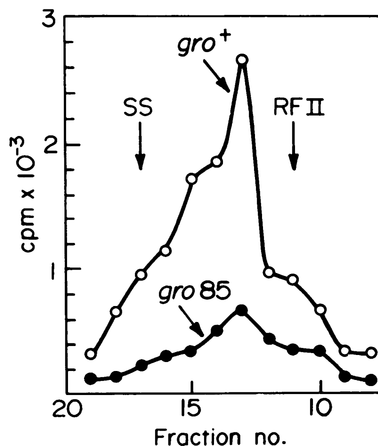

FIG. 5. *Synthesis of late RF in CM (30 μg/ml) after infection of the isogenic strains gro$^+$ and gro85 with φX174 wild type. Procedures were as for Fig. 1.*

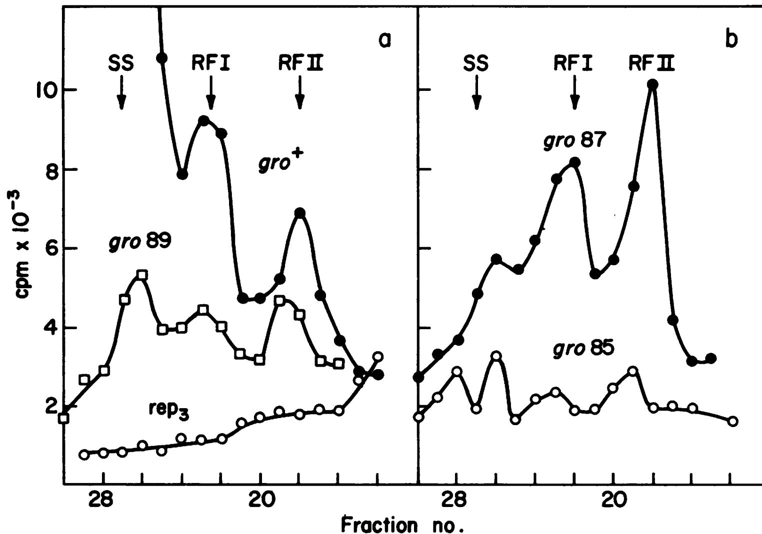

FIG. 6. *Formation of progeny RF by φX amE3 in the absence of CM in the isogenic strains gro^+, gro85, gro87, gro89, and rep_3. The cultures were infected as described in the legend to Fig. 1, [³H]thymidine was added 10 min after infection at 37°C, and the cultures were chilled 8 min later.*

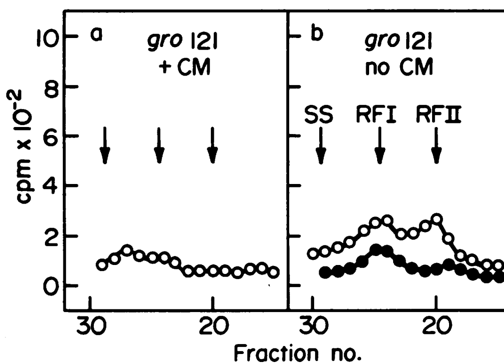

FIG. 7. *(a) Absence of formation of late RF by strain gro121 in CM (30 μg/ml). CM was added 3 min before infection; labeling was from 30 to 60 min after infection with φX am3 at a multiplicity of infection of 7. (b) DNA synthesis by strain gro121 in the absence of CM. Labeling was from −1 to 60 min. The lower curve shows the amount of parental RF formed in 150 μg of CM per ml after infection of gro85. (Extract centrifuged in same run.) In this experiment the SS peak formed by gro^+ reached 2×10^5 cpm.*

ability of the strain to produce progeny RF might be accounted for. Therefore, phage particle formation was measured in strain *gro*89 using the lysis mutant S13 *am*E as the infecting phage. Wild-type φX174 was also used in this experiment. Infection was in M9 medium at 37°C. In the case of the lysis mutant, a small amount of phage production is in fact observed (Fig. 8). However, this level of leakiness is much too low to account for the normal amount of progeny RF synthesis shown by *gro*89 in Fig. 1.

Infection with wild-type φX174 shows no sign of leakiness. The difference between the S13 *am*E curve and the φX wild-type curve in Fig. 8 is considered to be the result of delaying lysis and not due to differences between S13 and φX174.

Strain *gro*87, which also gives normal progeny RF synthesis (Fig. 1 and 6), was tested for particle yield with wild-type phage, rather than with lysis mutant, and gave an S13 burst of 1.4. The burst size of wild-type φX174 in *rep*40 is 1.3, and in *gro*85 it is 1.4.

Co-transduction with *ilv* of *gro*L⁻ and *rep*⁻. The frequencies of co-transduction with *ilv* were compared for strain *gro*87 and for rep_3. Isogenic donors and isogenic recipients were used. The co-transduction frequencies are the same within the error of measurement (Table 2). Thus, from the transduction data it is possible, but not proven, that the *gro*L⁻ mutants are mutants of the *rep* gene.

The co-transduction frequencies with *ilv* were 78% for rep_3 and 83% for *gro*87. Moreover, both *gro*87 and rep_3 are on the same side of *ilv*. It is known for rep_3 that the order is *ilv rep metE* (1). We find that *metE* is 10% co-transducible with rep_3 and 25% co-transducible with *gro*87. Thus, *metE* can be no further from *gro*L than from *rep*.

Dominance. gro^+/gro^- merozygotes were constructed by introducing the F′14 episome into the recipient strain *gro*87 *metE*. The pres-

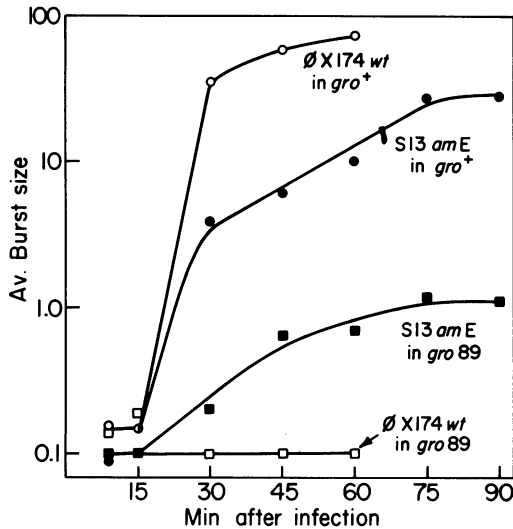

FIG. 8. *Determination of degree of leakiness. Cultures of gro+ and gro89 were grown to 2 × 10^8 cells/ml in M9 medium. $MgSO_4$ was added to 2 × 10^{-2} M, and then one-half of each culture was infected with φX174 wild-type at a multiplicity of infection of 4; one-half was infected with S13 amEn15 (lysis mutant). After 7 min of adsorption at 37°C, the cultures were diluted 10^3 times in M9 medium and allowed to grow for the designated times. Cultures infected with φX174 wild type were plated without artificial lysis; cultures infected with the S13 lysis mutant were plated after artificial lysis (31).*

ence of the episome was confirmed by the acquisition of maleness by the recipient and its conversion to *met*$^+$. The *gro*$^+$/*gro*$^-$ merozygote was *gro*$^+$ when used as indicator for plating φX174 wild type. Therefore, *gro*$^+$ is dominant to *gro*$^-$ and the *gro*$^-$ product is not inhibitory. It is known that *rep*$^+$ is dominant to *rep*$^-$ (1).

M13 growth. The filamentous small DNA phage M13, which requires the *rep* protein for growth, was tested on the *gro*L$^-$ strains. M13 forms plaques on male derivatives of the isogenic strains *gro*87 and *gro*89 with 100% EOP, but the plaques on *gro*87 are almost invisible. Strain *gro*85 was also tested and gave very small plaques. Therefore, M13 development involves the protein, which is mutated in these strains.

Other phages. *gro*L$^-$ strains permit the growth of λ, T4, T7, P1, and P2.

***ogr* mutants and the identity of *gro*L$^-$ with *rep*$^-$.** For most of the bacterial mutants that are P2 *gro*$^+$, φX *gro*$^-$, there arise spontaneous phage mutants (*ogr*) when high concentrations of φX174, S13, or G4 are plated, although the frequency and plaque type of the phage mutants obtained are very different for the various host strains. All *ogr* mutants grow on *E. coli* C. Five different mutant host strains could be distinguished on the basis of their pattern of yielding and plating *ogr* mutants and G phages. These are *gro*$^-$ strains *gro*8, *gro*40, *gro*85, *gro*87, and *gro*89. *ogr* mutants are named by the *gro*$^-$ strain on which they arise. Of the nine P2 *gro*$^+$ strains originally isolated, two strains could not yield or plate *ogr* mutants. One strain appeared to be identical to *gro*87 and one to *gro*89. The *ogr* yielders, *gro*87 and -89, are the *gro*L$^-$ strains in which phage development is blocked at a late stage. The three other strains, *gro*8, *gro*85, and *gro*40 (*rep*40), were found to be related to the *gro*L$^-$ strains from their pattern of *ogr* mutant growth (Table 3).

Strain *gro*40 has been shown by DNA analysis to be a *rep*$^-$ strain in that it forms no progeny RF. However, unlike all previously studied *rep*$^-$ strains, it permits phage *ogr* mutants (G4 *ogr*40) to arise and allows the plating of another *ogr* mutant (G4 *ogr*85). We assume that any two host strains are mutated in the same gene if the same phage *ogr* mutant grows on both. The mutation in the phage is producing an altered phage protein which can now interact with a specific altered host protein. Strain *gro*85 allows the plating of *ogr*40, *ogr*85, and *ogr*89 (Table 3). Strain *rep*40 would therefore be mutated in the same gene as *gro*85 and *gro*89 (which is *gro*L$^-$). We conclude that the *gro*L$^-$ mutations lie in the *rep* gene, although their

TABLE 2. *Co-transduction frequencies of a groL$^-$ mutation and a rep$^-$ mutation with ilv*[a]

Donor[b]	Transductant	Transduction frequency	
		No.	% of total
ilv$^+$ *rep*$_3$ *met*$^+$	*rep*$_3$	121	78
	rep$^+$	35	
	rep$_3$ *met*$^+$	10	10
	rep$_3$ *metE*	86	
ilv$^+$ *gro*87 *met*$^+$	*gro*$^-$	126	83
	gro$^+$	25	
	gro$^-$ *met*$^+$	24	25
	gro$^-$ *metE*	75	

[a] The recipient strain was C-1412 and the *rep*$_3$ donor was C-1415, both constructed by Calendar et al. (1). The *gro*L$^-$ donor, *gro*87, was isogenic with C-1412 and C-1415. Selection for *ilv*$^+$ colonies was on M9-minimal plates supplemented with methionine and histidine at 40 μg/ml. Transductant colonies were streaked on these same plates before being grown up in liquid culture for scoring for phage growth. Transduction and scoring are described in Materials and Methods.

[b] Recipient: *ilv*$^-$ *gro*$^+$ *rep*$^+$ *met*$^-$.

phenotypic effects are different from rep^- mutations.

The pattern of growth of wild-type ϕX174, S13, the G phages, and various *ogr* mutants is shown in Table 3. Each of the wild-type G phages is seen to be a naturally occurring *ogr* mutant for two gro^- strains. The G phages adsorb well to the gro^- strains on which they fail to plate.

Types of *ogr* mutants: *ogr*89 mutants. *ogr*89 mutants arose with a frequency of about 10^{-5} when wild-type S13 and ϕX174 were plated on strain *gro*89, appearing as very small, turbid, mottled plaques that segregate out large, clear plaques with high frequency. These secondary *ogr*89 mutants, which form large plaques, are consequently at least double mutants. Often the large plaques were also mottled, segregating out further large plaque-formers. Only large plaque-formers (the secondary or tertiary mutants) were used in burst size and rescue experiments. Both primary and secondary mutants were used in complementation tests for determining gene assignment. About 20 different ϕX *ogr*89 secondary plaque-type mutants were found; thus sites for mutation to *ogr* are numerous.

***ogr*87 mutants.** *ogr*87 mutants arose from S13 and ϕX174 on *gro*87 as a variety of small- and medium-sized plaque-formers with a frequency of about 10^{-10}. Some *ogr*87 mutants segregate out larger plaque-formers.

***ogr*40 mutants.** Only G4 produces *ogr* mutants on strain *gro*40. Phage G4 *ogr*40 arose as almost invisible specks on *gro*40 and formed a small plaque when replated on *gro*40. Plates must be incubated at 30°C or less.

***ogr*85 mutants.** *ogr*85 mutants arose from wild-type ϕX174, S13, and G4 on strain *gro*85 as almost invisible specks, with a frequency of about 10^{-8}. When replated on *gro*85 they formed small plaques. No G13 or G14 *ogr*85 mutants have been found. Despite the leakiness in progeny RF synthesis observed upon infection of *gro*85 by wild-type ϕX174 (Fig. 6), strain *gro*85 is a poor *ogr* yielder. Leakiness of a rep^- strain with respect to progeny RF synthesis is not an indicator of the ability to plate wild-type phage or to yield *ogr* mutants with high frequency.

DNA synthesis by an *ogr* mutant. S13 *ogr*89-1 showed the same amount of SS DNA synthesis in strain *gro*89 as in gro^+ by 23 min in M9 medium (data not shown).

Gene assignment of the *ogr* mutants. A search was made for *ogr* mutants that were also *ts* when plated on *E. coli* C. No *ts ogr* mutants were found for S13; however, for *ogr*89 mutants of ϕX174, about 25% of the large variety of secondary *ogr* mutants were *ts* and about 1% of all primary *ogr* mutants were *ts*. Complementation tests were carried out in *E. coli* C at 41.5 or 42.0°C to determine gene assignment. The results with one primary ϕX *ogr*89 mutant and

TABLE 3. *EOP of small DNA phages and their ogr mutants on gro^- and rep^- hosts*[a]

Phage	EOP on:						
	*gro*8	*gro*85	*gro*87	*gro*89	*rep*40	rep_3	*rep*121
S13	0	0	0	0	0	0	0
ϕX174	0	0	0	0	0	0	0
G4	0	0	1.0	1.0	0	0	0
G6	0	1.0	0	1.0	10^{-3}	0	0
G13	10^{-3}	0	1.0	0.25	0	0	0
G14	0	1.0	1.0	1.0	0	0	0
M13	0	1.0	1.0	1.0	0	0	0
S13 *ogr*89-1	0	1.0	1.0	1.0	0	0	0
*ogr*87-1	0	1.0	1.0	1.0	0	0	0
*ogr*89-85	0	1.0	1.0	1.0	0	0	0
ϕX *ogr*89-5	0	0	1.0	1.0	0	0	0
S13 *ogr*85	0	1.0	0	0	0	0	0
S13 *ogr*85-89	0	1.0	1.0	1.0	0	0	0
ϕX *ogr*85	0	1.0	0	0	0	0	0
G4 *ogr*85	0	1.0	0	0	1.0	0	0
G4 *ogr*40	0	1.0	0	0	1.0	0	0

[a] EOP on the gro^+ strain (C-1412) was 1.0 for all the phages. All host strains listed here except for rep_3 were P2 gro^+, ϕX gro^-. The strains designated *rep* form no ϕX progeny RF. Of the strains designated *gro*, strains *gro*87 and 89 are *gro*L$^-$; i.e., they form progeny RF in large amounts. Strain *gro*85 forms a small amount of progeny RF. Strain *gro*8 has not been analyzed for DNA synthesis. A zero indicates that the EOP on the given strain is less than 5×10^{-9}. However, in the case of strain *gro*89, *ogr* mutants arose from wild-type ϕX174 and S13 with a frequency of 10^{-5}. These mutants had an EOP of 1.0 when replated on *gro*89. G4 *ogr* mutant plates were incubated at 30°C.

two phenotypically different, secondary *ogr*89 mutants place the *ogr* mutants in gene F (Table 4).

ϕX *ogr*89-5 complements with mutants of all S13 genes except F; ϕX *ogr*89-105 and ϕX *ogr*89-1 complement with mutants of all genes except C and F. Lack of complementation between the C mutant of phage S13 and ϕX *am*D is, however, also observed. Lack of complementation with C is probably due to incompatibility of interaction between several mutated proteins of the two phages rather than being a true noncomplementation result. The positive complementation of the C mutant with ϕX *ogr*89-5 was observed in two experiments. It was also found that S13 *am*F61 was rescued well by ϕX *am*E3. Complementation tests with one other ϕX *ogr*89 primary and one other secondary mutant also showed no complementation with F and poor complementation with C, whereas complementation with mutants of all the other genes was good.

cis **dominance of the** ***ogr*** **mutations.** The burst sizes and rescuing ability of two S13 *ogr* mutants were compared in the *gro*$^+$ and *gro*89 strains. The *ogr* mutants used give good burst sizes in both *gro*$^+$ and *gro*89. Therefore, *gro*89 was used for the rescue experiments. (*ogr* burst sizes are low in other *gro*L$^-$ strains, being about 10 for *ogr*87-1 in *gro*87.) The unexpected result obtained was that *ogr* mutants give very poor rescue of non-*ogr* mutants in the *gro*$^-$ strain (Table 5) but good rescue in the *gro*$^+$ strain. (The non-*ogr* mutants used were all amber mutants, for convenience in distinguishing genotypes.) Thus, the *ogr* mutants are *cis* dominant in their ability to grow on the *gro*$^-$ strain. It was quite unexpected that mutations located in the gene coding for the diffusible F capsid protein should be *cis* acting.

A relatively high rescue of gene A amber mutants in strain *gro*89 was found. It has long been known that rescue of gene A mutants is poor (34), indicating that the gene A protein is *cis* acting. A *gro*$^+$ strain was the host in those experiments. Rescue of several non-*ogr* mutants is good in *gro*$^+$ and very poor in the *gro*$^-$ strain. Therefore, it was expected that *am*A mutants, which already have the handicap of poor rescue in *gro*$^+$, would show a further large decrease in *gro*$^-$. Instead, the burst size of *am*A mutants was the same low value in both the *gro*$^+$ and *gro*$^-$ strains. This result was obtained with two different *am*A mutants (Table 5).

***ogr*-interfering mutations in genes A, F, and H.** Some *ts* mutations in S13 interfere with the *ogr* property. The procedure used is to obtain *ogr*89 derivatives of *ts* mutations in each of seven phage genes. About 10^8 particles of a well-characterized *ts* mutant are plated on

TABLE 5. *Poor rescue by phage S13 ogr mutants of non-ogr phage in a gro$^+$ host and a gro$^-$ host*[a]

Non-*ogr* phage	Burst size of non-*ogr* phage after unmixed infection in *gro*89	Burst sizes after mixed infection in:			
		*gro*89 host		*gro*$^+$ host	
		*ogr*89-1 phage	Non-*ogr* phage	*ogr*89-1 phage	Non-*ogr* phage
*am*A105	0.10	37	4.3	236	4.5
	0.13	53	2.0	296	3.0
*am*B129	0.16	23	2.0	75	57
		63	6.5	87	50
*am*E15	1.0	84	7.0	167	59
amF28	0.20	28	1.0	29	41
*am*H66	0.08	39	2.0	53	30
		99		247	
		105		170	
*am*A105	0.20	51[b]	2.8	87[b]	2.4
*am*A113	0.38	21[b]	3.0	38[b]	3.6
*am*H66	0.08	42[b]	0.8	43[b]	41
		70[b]			

[a] The *ogr* mutants used were S13 *ogr*89-1 and S13 *ogr*87-1. The non-*ogr* mutants were S13 amber mutants, unable to grow in *gro*$^+$ (C-1412) since it is a nonsuppressing strain.

[b] *ogr*87-1 phage.

TABLE 4. *Burst sizes from complementation tests of ϕX174 ogr mutants versus standard mutants*[a]

ϕX174 *ogr* mutant	Burst size	Burst size of standard mutant:									
		*am*A105	*am*B129	*ts*C17	*am*D56	*am*E15	*am*F61	*am*F28	*am*G43	*am*H66	*am*H55
		0.2	0.01	0.01	0.10	0.02	0.23	0.15	0.17	0.12	0.06
*ogr*89-5	0.10	2.4	2.5	2.0	2.3	5.5	0.38	NT[b]	1.5	1.5	NT
*ogr*89-1	0.27	1.2	4.0	0.6	5.4	4.4	0.40	0.46	2.6	1.6	NT
*ogr*89-105	0.30	2.3	2.2	0.24	2.5	4.7	0.26	0.22	3.2	NT	1.6

[a] The standards used were S13 mutants except for *am*D56, which is a ϕX174 mutant. The first row gives the burst sizes obtained from unmixed infection with the standard mutants. These have not been subtracted from the mixed infection. The first column gives the burst sizes obtained from unmixed infection with the ϕX174 *ogr* mutants. Complementation was carried out in broth-grown *E. coli* C1a at 42.0C.

[b] NT, Not tested.

*gro*89 indicator at the permissive temperature (35°C). The plaques that appear are *ogr*89 *ts* mutants, and in most cases they arise with a frequency of about 10^{-5}. However, five phenotypically different *ts*A mutants yielded no *ogr* mutants ($<10^{-8}$). One gene F mutant also yielded no *ogr* mutants.

Another *ts*A mutant gave rise with normal frequency to an *ogr*89-*ts*A mutant, but its *ogr*89 character was altered. *ogr*89 mutants plate with 100% EOP on strain *gro*87 at 35°C and on strain *gro*89 at 39°C, but the *ogr*89-*ts*A mutant in question could not form plaques under these conditions. Two *ts* mutants in gene F and one in gene H also affect *ogr*89 in the same way, but no mutants of this type have been found in genes B, C, D, or G. It should be noted that more gene A mutants were available for testing than mutants of other genes. The numbers of mutants tested were 13 in gene A, 3 in B, 2 in C, 1 in D of ϕX174, 5 in F, 3 in G, and 5 in H. It is possible that other mutants in genes B, C, D, and G might have *ogr*-interfering properties.

Temperature dependence of *ogr* mutant growth in gro^- hosts. Although numerous *ogr* mutants have been isolated on the gro^- strains studied here, none of these mutants can yield phage on the gro^- strains at high temperature (42.0°C). The gro^- strains alone grow well at high temperature, and the *ogr* mutants grow well on the gro^+ strain at high temperature. The amount of late RF synthesis by phage *ogr*89 in 30 μg of CM per ml is the same in gro^+ and *gro*89 at 42.0°C. Thus, the block to *ogr* mutant growth in a gro^- strain at high temperature occurs late in infection. This temperature dependence of *ogr* mutant growth provides a means of shifting from a gro^- to a gro^+ state, or the reverse, during the course of phage infection.

Properties of the *gro*L strains. So far no striking defect has been detected in the transduced *gro*L^- strains other than the *gro*L^- property. Both show the same growth rate as the isogenic gro^+ strain, C-1412. gro^+ and *gro*89 are retarded in growth to the same extent by 2% sodium deoxycholate, so presumably no membrane defect is involved. *gro*89 is slightly more UV sensitive than gro^+. The UV survival curves for the isogenic strains *gro*89 and gro^+ (C-1412) are shown in Fig. 9. Calendar et al. (1) obtained a similar result in a comparison of the rep_3 strain isogenic with C-1412.

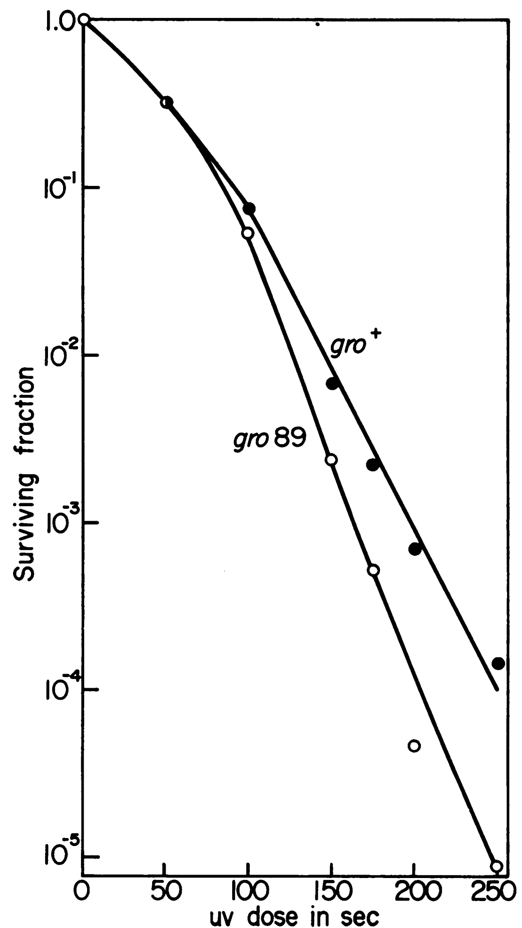

FIG. 9. *UV sensitivity of the isogenic strains* gro^+ *(C-1412) and gro89. Cultures were grown in M9 medium to* 2×10^8 *cells/ml and diluted 10 times in cold M9 salts medium, and 2-ml samples were irradiated at 45 cm from a 15 W germicidal lamp for the designated times. Samples were diluted into cold M9 salts medium and plated in the dark.*

DISCUSSION

We report here that a step late in the development of small DNA phages requires the interaction of a phage protein with a host protein. The host protein is the product of a gene defined by the *gro*L^- (late block) class of bacterial mutations. This host protein appears to be identical with the *rep* protein, previously shown to be needed early in infection (5) and now seen to be needed late also. It is non-essential for cell viability. The phage protein in this complex is the F protein, the major protein of the phage coat. *gro*L^- mutants block growth of the related small, icosahedral, SS phages S13 and ϕX174 and the recently isolated (13) ϕX-related phage G6. *gro*L^- mutants also affect adversely, but do not block, the growth of the filamentous phage M13. In this case no evidence of a host-phage

protein interaction has yet been shown. *gro*L⁻ mutants permit the growth of λ, T4, T7, P1, and P2.

Wild-type S13 and φX174 are unable to grow on the *gro*L⁻ strains but can spontaneously mutate to overcome this host block. The phage mutations are located at numerous sites in gene F. One model to explain the growth of these phage mutants (*ogr* mutants) on *gro*⁻ strains is the formation of a complex between a host protein and a phage protein. According to this model, when the host protein is altered in conformation by mutation, the phage protein must receive a compensating alteration to achieve a proper fit. It seems likely that this type of interaction may be taking place between the phage F protein and the host *rep* protein. In order for *ogr* mutants to grow well on a *gro*L⁻ host, two and often three phage mutations appear to be necessary, each successive mutation giving improved growth.

In the present work, examination of phage DNA synthesis showed that two *gro*⁻ host mutants (*gro*87 and -89) form normal amounts of progeny RF synthesis in CM (30 μg/ml). However, they form no stable SS. Therefore, it is assumed that these two *gro*⁻ mutants, now designated *gro*L⁻ (late block), are blocked in either SS synthesis or phage maturation, or both. In the case of the small DNA phages, it is difficult to distinguish between blocks at these different stages of development, because SS is never found in the form of free DNA in cell (*gro*⁺) extracts but only in mature phage particles (31, 39). It is possible that for these phages SS synthesis and phage maturation are interdependent.

A third *gro*⁻ strain gave unexpected results. This strain, *gro*40, which like *gro*87 and *gro*89 is φX *gro*⁻, P2 *gro*⁺, permits the growth of phage *ogr* mutants. However, wild-type phage formed no progeny RF in *gro*40, and this strain is therefore, by definition, designated a *rep*⁻ mutant, *rep*40. The strain is a good adsorber and is negligibly leaky. It is the first example of a *rep*⁻ strain that permits phage mutant growth.

A fourth *gro*⁻ strain, *gro*85, formed a small amount of progeny RF in either the presence or absence of CM. It is classified as a leaky *rep*⁻ strain and it is also an *ogr* yielder. Despite its leakiness, it is of considerable interest because its *ogr*-plating properties show that the *gro*L⁻ strains are related to strain *rep*40.

The relationship between *rep*⁻ and *gro*L⁻ is inferred from the fact that the same phage mutations enable phage to grow on both types of cell. The use of phage mutant growth as an indicator of a genetic relationship between host strains is based on the assumption that a phage protein has mutated to interact with a specific altered host protein. Phages *ogr*40 and *ogr*85 grow on a *rep*⁻ strain, *rep*40; phages *ogr*89, *ogr*40, and *ogr*85 grow on strain *gro*85; phage *ogr*89 grows on strain *gro*89 (which is *gro*L⁻). Thus, all three host strains must be mutated in the same gene, the *rep* gene.

The late stage in the development of φX-related phages is not well understood, but it is known to involve the interaction of several phage-coded proteins. (Part of this subject is included in Denhardt's intensive review [4] of the small DNA phages.) It has long been known that the products of genes B, C, F, G, and H are needed to yield infectious SS DNA (35-37). It was shown that mutants in genes B, D, F, and G yielded no SS DNA at all (22), but gene H mutants do form SS DNA (6, 22, 30) which is noninfectious (30).

Iwaya and Denhardt (16) investigated the block in SS DNA synthesis shown by mutants in genes B, D, F, and G of φX174 and found by pulse labeling that there is a true block in DNA synthesis with these mutants rather than synthesis followed by degradation. These authors concluded that SS synthesis is not initiated in these mutants. The gene A product is needed for SS synthesis (Tessman and Peterson, unpublished data) as well as for progeny RF synthesis (35). Functions are known for each of the phage gene products except for C and J. Evidence from two sources (7, 16) indicates that the function of the φX D protein is different from the function found (28) for the gene 5 protein of M13, i.e., protection of SS from conversion back to RF. The A* protein (23) may be a capsid component (4) and also appears to be required for shut-off of host DNA replication (25). Multifunctional proteins seem to be common among the small icosahedral DNA phages. Tonegawa and Hayashi (38) described the protein aggregates of F and G, which are precursors to the mature capsid. Siden and Hayashi (29) showed that F and G proteins are complexed into 12S multimers through the catalytic action of the B protein and that these F-G complexes become part of the capsid, although B does not. Presumably, D protein associates with SS as it is being replicated. The F-G complexes would then have to displace D protein from the DNA. Evidence that there must be recognition between the F and D proteins comes from the fact that S13 D protein and φX174 F protein are unable to interact to produce viable phage, as shown by lack of complementation (19) between φX *am*D and S13 *am*F mutants.

Weisbeek and Sinsheimer (39) have described a structure that is a possible intermedi-

ate in ϕX particle formation. This structure contains SS DNA and capsid proteins F, G, H, and J in normal amounts, plus large amounts of D protein.

The H protein must also bind to the SS DNA, since of all the phage proteins H protein alone enters the host cell together with SS DNA in the next cycle of infection (17, 18). It is likely that H protein must also displace D by a protein-protein interaction.

The role of the presumptive *rep*-F complex in this scheme of ϕX SS DNA synthesis and particle maturation is still unknown. It is likely that the late-acting complex is in fact a ternary complex consisting of the *rep*, F, and A proteins. Evidence that the A protein interacts with the *rep* protein is that a number of different mutants in gene A are unable to give rise to *ogr* mutant derivatives; i.e., certain combinations of an A mutant and an F mutant in the same genome are not viable in the gro^- cell. It is possible that an *ogr* mutation either in gene A or F could compensate for the mutation in *rep*. So far, five out of five *ogr* mutants tested have been F mutants, however.

The fact that an *ogr* mutant can overcome the block in a *rep* strain, which by definition is blocked in progeny RF synthesis, suggests that an F-*rep* complex must normally be required for formation of progeny RF. However, it is known that the F protein is not needed for formation of progeny RF (22, 35). How, then, can a mutation in phage gene F overcome the host *rep* block? The growth of *ogr* mutants in strain *rep*40 might be explained by assuming that a *rep*-F complex is normally used in the formation of progeny RF, but if F protein is missing, progeny RF can still be synthesized by some bypass mechanism. Defective or missing *rep* protein cannot be tolerated, however. An *ogr* mutation in F compensates for a mutated *rep* protein and permits progeny RF synthesis.

Another hypothesis to explain how a phage mutation in gene F can permit phage growth on a rep^- strain is that F protein is not involved in progeny RF synthesis but interacts with *rep* protein to form a complex that acts only at the stage of SS synthesis. According to this model, a rep^- strain would have to be at least slightly leaky in progeny RF synthesis to permit the growth of a phage *ogr* mutant. The burst size of a phage *ogr* mutant in a rep^- strain would then be limited by the amount of progeny RF available for the F*-*rep** complex to act upon. (The asterisks denote mutated proteins.) This hypothesis is designed to explain the fact that progeny RF is made in the absence of F protein.

A more complex explanation for the growth of phage *ogr* mutants in rep^- strains is that an early-acting *rep* complex might consist only of the *rep* and A proteins, whereas the late-acting complex includes the F protein too. It is necessary to assume again that a mutation either in gene F or A can overcome the gro^- block to yield an active early-acting *rep*-A complex that allows progeny RF formation in the rep^- strain. At present it is not technically feasible to determine the gene location of the two G4 *ogr* mutants that grow on strain *rep*40, so these assumptions cannot be tested.

The *cis*-limited action of the presumptive F-*rep* complex is not understood. The action is apparently *cis* in the gro^- (*rep*) strain but *trans* in the gro^+ (rep^+) strain. This assumption is based on the fact that *am*F mutants can be rescued in a gro^+ strain; since *am*F mutants form no F protein, they must obtain their F-*rep* complex by diffusion from the rescuing phage.

The present work demonstrates that the *rep* protein, in addition to phage proteins A, B, C, D, F, G, and H, is involved in phage SS DNA synthesis or maturation. The apparent identity of rep^- mutations with $groL^-$ mutations indicates that the *rep* protein is needed both early for progeny RF synthesis and late for SS synthesis in the development of ϕX174-related phages.

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