Mutations in Coliphage P1 Affecting Host Cell Lysis

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A total of 103 amber mutants of coliphage P1 were tested for lysis of nonpermissive cells. Of these, 83 caused cell lysis at the normal lysis time and have defects in particle morphogenesis. Five amber mutants, with mutations in the same gene (gene 2), caused premature lysis and may have a defect in a lysis regulator. Fifteen amber mutants were unable to cause cell lysis. Artificially lysed cells infected with five of these mutants produced viable phage particles, and phage particles were seen in thin sections of unlysed, infected cells. However, phage production by these mutants was not continued after the normal lysis time. We conclude that the defect of these five mutants is in a lysis function. The five mutations were found to be in the same gene (designated gene 17). The remaining 10 amber mutants, whose mutations were found to be in the same gene (gene 10), were also unable to cause cell lysis. They differed from those in gene 17 in that no viable phage particles were produced from artificially lysed cells, and no phage particles were seen in thin sections of unlysed, infected cells. We conclude that the gene 10 mutants cannot synthesize late proteins, and it is possible that gene 10 may code for a regulator of late gene expression for P1.

A genetic map for P1 (Fig. 1), consisting of 113 amber mutations, has been established (31) using deletion prophages, two-factor spot tests, and three-factor crosses. The two-factor crosses permitted assignment of the mutations into 10 linear arrays or linkage clusters, I through X, with mutations in cluster X behaving as two subgroups, X-1 and X-2. However, cistron designations, apart from those originally assigned by Scott (22), were not assigned because complementation tests in liquid frequently gave ambiguous results.

With other phages, amber mutants can be divided into two major classes depending on whether they have a gene defective in a function which is required early or one which is required late in phage particle development ("early" and "late" genes). Late genes are involved in particle morphogenesis (e.g., production of proteins which become structural components of the particle) and cell lysis (e.g., genes R and S in λ ; 9, 10). Mutants having a defect in late genes involved in particle morphogenesis can lyse the host cell under nonpermissive (*sup*⁰) conditions, whereas those having a defect in a lysis function cannot. Early genes are involved in regulation of transcription, DNA synthesis, and recombination (e.g., genes N, O, P, and Q in λ). In addition, mutations in genes such as N, O, P, and Q do not allow synthesis of any of the late gene products of λ , including those effecting lysis. Therefore, to help determine which P1 amber mutants are defective in early genes and which are defective in late genes, we have tested 103 P1 amber

mutants for their ability to lyse *sup*⁰ cells. Those mutants that lyse *sup*⁰ cells would probably have a defect in late genes involved in morphogenesis, whereas those that do not lyse *sup*⁰ cells would probably have an early gene defect or a defect in a late gene determining a lysis function.

MATERIALS AND METHODS

Bacterial and phage strains. Bacterial strains are described in Table 1. Phage strains are P1*vir* (the supervirulent mutant P1*kcvir*^r of Sarkar which grows on strains lysogenic for P1; 22); P1*vir* amber mutants (Fig. 1) 1, 2, 4, 6, 7, 11, 13, 14, 16, 17, 19, and 21 of Scott (22) and 91 *vir* amber mutants of Walker and Walker (30) (for simplicity these mutants will be referred to as *am*1, etc., without the *vir* designation); T6 and λ CI857*Sam*7 (gifts from M. Howe); and λ CI857*Ram*5 and λ CI857 (gifts from M. Feiss).

Media, phage assays, and preparation of phage stocks. These were the same as described previously (30), except that L-broth (1% Trypticase [BBL Microbiology Systems], 0.5% yeast extract [Difco], 1% NaCl, 0.1% glucose, and pH adjusted to 7.4 with 1 N NaOH) was used instead of WLB. In experiments where cells were infected with λ wild-type or mutant phages, cells were grown in L-broth containing 0.1% maltose and 10 mM MgSO₄ instead of 0.1% glucose. Streptomycin (streptomycin sulfate; Pfizer Laboratories) was used at 10 μ g/ml; spectinomycin (spectinomycin hydrochloride; Upjohn) was used at 40 μ g/ml; and tetracycline (tetracycline hydrochloride; Lederle) was used at 10 μ g/ml.

Lysis of phage-infected cells. At 0 min the desired amber mutant was added (at a multiplicity of infection [MOI] of 4 to 6 except where stated otherwise) to a log-phase L-broth culture of *Shigella dysen-*

FIG. 1. Genetic map of P1 amber mutations, modified from Walker and Walker (31). Regions demarcating the 10 linkage clusters are represented by solid blocks. The cistron designations above the map line include the original 1 to 10 of Scott (22). Other designations are *vir* and eight *c* genes (22–25; J. R. Scott, unpublished data). The orientation of groups of amber mutations within parentheses or within brackets with respect to mutations outside these groups is unknown. Since this map was first published, the orientation of the group of mutations in linkage cluster I and of the group of mutations, 127 through 75, in linkage cluster IV has been established (J. R. Scott, R. Mural, and D. Vapnek, personal communication) by three-factor crosses and by rescue of *am*⁺ alleles from cloned P1 DNA fragments which had previously been mapped by restriction endonuclease analysis (1). In addition, rescue tests with such cloned fragments has established that the order of the subgroups X-1 and X-2 and the orientation of subgroup X-1 is as shown (Sternberg, as cited in reference 32). The spacing of the hash marks is not meant to indicate either genetic or physical distance. Amber mutations that are underlined denote those mutants that were not used in the experiments reported in this paper.

TABLE 1. Bacterial strains

Strain designation ^a	Relevant genotype ^a	Source or reference
Sh-15	<i>sup</i> ⁰	Sh (2)
Sh-16	Sh-15 <i>str</i>	Sh/s; (2)
Sh-16 <i>supF</i>	Sh-16 <i>supF tet</i>	M. Van Montagu
DW101	<i>sup</i> ⁰	(30)
DW103	DW101 <i>supD</i>	(30)
DW104	DW101 <i>str</i>	This laboratory
DW106	DW101 <i>str tsx</i>	This laboratory
DW107	DW101 <i>str tsx spc</i>	This laboratory
DW304	DW103 <i>str</i>	This laboratory
DW306	DW103 <i>str tsx</i>	This laboratory
DW307	DW103 <i>str tsx spc</i>	This laboratory
R594	<i>sup</i> ⁰ <i>str</i>	(3)
MF380	R594(<i>Ac1857Aam32</i>)	M. Feiss

^a Abbreviations and genotypes: Sh-15 and Sh-16, strains of *S. dysenteriae*; DW strains, derivatives of *E. coli* K-12 *sup*⁰, nonpermissive for amber mutants; *supD* and *supF*, suppressors of amber mutations; *str*, streptomycin resistance; *spc*, spectinomycin resistance; *tet*, tetracycline resistance; *tsx*, resistance to phage T6.

*teriae sup*⁰ strain Sh-16 or Sh-15, or *Escherichia coli* K-12 *sup*⁰ strain DW101. *vir* amber mutants were used to avoid lysogenization (15). After allowing 10 min at 37°C for adsorption in the presence of 5 mM CaCl₂,

the culture was diluted 2.5-fold in fresh medium containing 5 mM MgSO₄ and incubated at 37°C with vigorous shaking. The optical density of the culture was read at 5- to 10-min intervals at a wavelength of 600 nm in a Bausch and Lomb Spectronic 20 spectrophotometer. An uninfected culture and a culture infected with P1*vir* were included as controls. Cultures that did not lyse were assayed for viable cells. In experiments where burst sizes were measured, infected Sh-15 or DW101 cultures were washed by filtration after the 10-min adsorption period, to get rid of unadsorbed phage, and progeny phage were assayed on *sup*⁰ and *sup*⁺ strains Sh-16 and Sh-16*supF* or DW104 and DW304.

Artificial lysis of phage-infected cells by using phage T6. Cells of *E. coli* K-12 *sup*⁰ strains DW101 or R594 were infected with amber mutants or with P1*vir* (MOI, ~5). After 10 min at 37°C, the cells were washed on a Millipore membrane filter, diluted 1:1 in fresh L-broth, and incubated at 37°C until 80 min (unless stated otherwise) after infection. Cultures were divided, and T6 was added to one sample (MOI, ~500) and P1 buffer was added to the other. After incubation at 37°C for 15 min, cultures which previously had not lysed showed visible lysis where T6 had been added. Samples from preparations which had and had not been treated with T6 were assayed on the *tsx supD*

and *tsx sup*⁰ strains DW306 and DW106 or DW307 and DW107. The burst sizes on DW306 and DW307 were normalized to the burst size of P1*vir*, set at 100.

Electron microscopy of sectioned, phage-infected cells. Phage-infected *E. coli* DW101 cells were prepared as in the method for studying lysis of phage-infected cells. At 55 min after infection for *vir*-infected cells, and at 110 min after infection for amber mutant-infected and uninfected cells, samples were prepared by either of the following methods.

Method 1. Culture samples (3 ml) were added to 1 ml of 8% aqueous glutaraldehyde (at 37°C) and incubated for 30 min at 37°C for prefixation. Each sample was centrifuged for 1 min in a Beckman microfuge, and the pellet was covered with 0.5 ml of 1% OsO₄ in 0.2 M phosphate buffer for 1 h at room temperature for the main fixation. The pellet was rinsed in distilled water and placed in a small glass vial, and 2.5% (wt/vol) aqueous uranyl acetate was added for 2 h for postfixation. The pellet was rinsed in distilled water, dehydrated with 70% acetone and 100% acetone (10 min each), and infiltrated with Spurr resin-acetone solutions of 1:1 for 30 min and 100% Spurr resin for 45 min. Polymerization was at 65°C for 2 days.

Method 2. Culture samples (3 ml) were added to 1 ml of 8% aqueous glutaraldehyde (37°C) containing 0.01 M CaCl₂ and incubated for 30 min at 37°C for prefixation. After centrifugation in a Sorvall table-top centrifuge for 10 min, each pellet was covered with a mixture of 1 ml of 1% OsO₄ in RK buffer (0.01 M CaCl₂, 0.117 M NaCl, Michaelis buffer, pH 6.1; 14) and 0.1 ml of L-broth and left at room temperature overnight for the main fixation. RK buffer (8 ml) was added, and the preparation was centrifuged for 10 min. The pellet was postfixed with 1% uranyl acetate in RK buffer for 3 h. After centrifugation for 1 min in a microfuge, the pellet was rinsed twice with distilled water and dehydrated with 70% acetone (10 min) and 100% acetone (overnight). Embedding was by infiltration with Epon-araldite-acetone (1:1) for 1.5 h, Epon-araldite-acetone (3:1) for 2.5 h, and 100% Epon-araldite for 30 min. Polymerization was at 65°C for 2 days.

Thin sections were cut with a nominal thickness of 30 nm with an LKB microtome, using a diamond knife. Sections were stained in 5% (wt/vol) aqueous uranyl acetate. A JEOL 100B electron microscope was used with an anticontamination device. The acceleration voltage was 60 kV, and a 40-μm thin foil objective aperture was used. Micrographs were made on Kodak 4463 film.

RESULTS

Lysis of cells infected with amber mutants. The ability of 103 P1 amber mutants to cause lysis of nonpermissive cells was measured by following the optical density of phage-infected *S. dysenteriae* Sh-16 or Sh-15 cells. *S. dysenteriae* cells were used rather than those of *E. coli* because, in future work, lysates will be examined by electron microscopy for phage structures, and lysates of shigella cells give less debris (e.g., flagella), which would obscure phage structures, than do *E. coli* lysates. Under the conditions used, lysis (as indicated by a drop in

optical density) of the control culture infected with P1*vir* commenced between 60 and 70 min after infection and was complete by 110 min (Fig. 2).

Cell lysis was observed with 88 of the 103 *vir* amber mutants (Fig. 1) tested. These 88 mutants consist of all of those mutants tested in linkage clusters I, II, IV, V, VI, VII, VIII, IX, and X-1. Of these 88 mutants, 83 gave results (e.g., *am*139; Fig. 2) similar to the result shown for P1*vir*. With the remaining 5 of the 88 amber mutants, lysis occurred 20 to 30 min before such lysis occurred with P1*vir* (premature lysis; e.g., *am*31; Fig. 2). These five mutations are in linkage cluster IV and have been shown to be in the same cistron by complementation tests (31). They are designated as mutations 17, 31, 56, 103, and 170 in cistron 2 (J. R. Scott's designation; 22).

These five mutants did not show premature lysis after infection of the Sh-16*supF* isogenic host, indicating that the premature lysis phenotype is due to the amber mutations, which are suppressed in the *supF* host. When Sh-15 (*sup*⁰) was coinfecting with P1*vir* (MOI, 5) and one of these mutants (*am*56; MOI, 5), lysis occurred at an intermediate time compared to when lysis occurred with cells infected separately (MOI, 10) with these phage strains (Fig. 3). Under these conditions, the burst size (46 PFU/cell) obtained after coinfection was closer to that obtained by single infection with P1*vir* (286 PFU/cell) than

FIG. 2. Lysis of *S. dysenteriae* Sh-16 (*sup*⁰) cells after infection with P1*vir* (○), *am*20 (●), *am*175 (□), *am*139 (■), and *am*31 (△). (▲) Growth of uninfected cells. *am*20 and *am*175 did not show significant lysis even when the time after infection was extended to 180 min.

FIG. 3. Lysis of *S. dysenteriae* Sh-16 (sup^0) cells after infection with P1vir (○), am56 (●), or P1vir + am56 (△).

to that obtained with am56 infection (0.6 PFU/cell). (A second experiment gave similar lysis curves, with burst sizes of 26, 114, and 0.5 PFU/cell.) From these results we conclude, by analogy with results from use of phages such as λ (18), that the am^+ allele is dominant to the am56 allele in permitting relatively normal lysis and burst sizes to occur in mixed infection. The intermediate lysis time obtained in mixed infections may be due to a gene dosage effect and may itself be responsible for the intermediate burst sizes. Of the progeny from the mixed infection, 64% were amber phage, which suggests that, under these conditions, am56 DNA is synthesized and packaged at least to the same extent as the am^+ DNA.

Fifteen of the 103 amber mutants tested did not show lysis of sup^0 *S. dysenteriae* cells. These 15 mutations are located in two groups on the P1 genetic map. They are the 5 mutations comprising linkage cluster III (e.g., am20; Fig. 2) and the 10 mutations comprising linkage cluster X-2 (e.g., am175; Fig. 2). In complementation tests for lysis performed with pairs of these 15 mutants (data not shown), lysis only occurred when the sup^0 cells had been coinfecting with a mutant from each of the two linkage clusters, suggesting that the 5 mutations in cluster III belong to one cistron and the 10 mutations in cluster X-2 belong to another.

Lysis in sup^0 *E. coli* K-12 cells was compared with that obtained with sup^0 *S. dysenteriae* cells because, in experiments described below, artificial lysis of cells infected with mutants from clusters III and X-2 was attempted, using methods described for *E. coli* strains infected with λ (10) or T4 (5). When representative mutants causing cell lysis (am139), premature cell lysis (am31, am56), or no lysis (am20 and am144 from cluster III; am111 and am175 from cluster X-2)

of *S. dysenteriae* sup^0 cells were used to infect *E. coli* K-12 strain DW101, results (Fig. 4) similar to those described for shigella cells (Fig. 2) were obtained. A difference observed was that, where there was lysis (i.e., with am31, am56, am139, and the P1vir control), it commenced 10 to 20 min earlier for each phage strain with DW101 than with Sh-15 or Sh-16. Assays for surviving cells at 40 min after infection of cultures with am20 or am175 showed that 94 to 96% of infected cells had not survived infection. This suggests that infection with mutants from either of the two linkage clusters is lethal to the cells.

Artificial lysis of phage-infected cells. To distinguish whether the lysis deficiency of the 15 mutants was due to an early gene defect or was due solely to a defect in a late gene determining a lysis function, various mutants from each of the two linkage clusters (III and X-2) were tested for their ability to produce PFU after artificial lysis of infected *E. coli* K-12 cells.

Phages in general have various gene products that cause, or help to cause, cell lysis. For simplification, and because they turned out to be sufficient for comparison of results with P1, we will confine ourselves to discussion of the λ lysis

FIG. 4. Lysis of *E. coli* K-12 strain DW101 (sup^0) cells after infection with P1vir (○), am20 (●), am111 (□), am31 (■), or am139 (△). (▲) Growth of uninfected cells. Mutants represented by numbers in parentheses gave curves similar to those obtained for mutants represented by the adjacent numbers.

genes. With phage λ , there are three known genes, R, S, and Rz (9, 10, 33), whose products are required for cell lysis. Those that have been studied most are genes R and S. Gene R codes for an endolysin with endopeptidase activity (28); under nonpermissive conditions, amber mutants of gene R cannot cause cell lysis, but phage particle production ceases at the time after infection when lysis would normally occur. Addition of an exogenous lytic enzyme such as lysozyme effects lysis of cells infected by R amber mutants. The gene S product is thought to alter the cytoplasmic membrane so that λ endolysin can act on its substrate in the cell wall (19). As with R amber mutants, S amber mutants cannot cause cell lysis. However, with S amber mutants, phage particle production continues to increase (up to 500 to 1,000 particles per cell; 9, 10) after lysis would normally occur. With S amber mutants, artificial lysis of infected cells is effected by addition of chloroform rather than lysozyme. Less is known about the recently described Rz gene. Rz⁻ mutants cannot cause cell lysis. However, Rz⁻-infected cells are transformed from rods to fragile spheres at the time lysis would normally occur, and it has been suggested that the Rz gene may code for a lysozyme-type protein which would cleave a bond in the cell wall peptidoglycan different from that cleaved by the R gene endolysin (33). It is not known whether Rz⁻ mutants continue to produce particles after the time of normal lysis. Because so little is known about the Rz function, we will compare our results obtained with lysis-defective P1 mutants with those obtained with the R and S mutants of λ .

Based on methods used by Harris et al. (10) and Edgar and Wood (7) with *E. coli* K-12 strains, various combinations of chloroform, lysozyme (0 to 1.6 mg/ml), EDTA (0 to 3.0 mM), freezing and thawing, and different times of treatment were used with DW101 cells infected with various P1 amber mutants, at 80 min after infection. Cultures infected with P1vir and untreated phage-infected cultures were included as controls. These methods were unsatisfactory because they resulted either in incomplete lysis of the phage-infected cells or in phage inactivation. There was no increase in burst size with any of these methods when cells were infected with mutants from linkage cluster X-2. However, when cells were infected with linkage cluster III mutants, small increases in burst size were obtained with some of the methods, the greatest increase being 20-fold, from 0.6 to 12 PFU/cell (using a 30-min treatment with 0.3 mM EDTA and 0.4 mg of lysozyme per ml).

As an alternative to chemical and physical methods, a biological means of lysing the P1-

infected cells was attempted, namely, induction of a λ *Aam sup*⁰ lysogen (MF380) infected with various P1 phages. An isogenic nonlysogen (R594) infected with the same phage was used as a control. Under these conditions, the burst sizes (Table 2) of P1vir and of *am136* grown on the λ lysogen were decreased 12- and 6-fold (from about 84 and 0.5), respectively, compared to their burst sizes on the nonlysogen; burst sizes of mutants in cluster III were increased up to 36-fold (from an average of 0.14), and burst sizes of mutants in cluster X-2 were increased up to 3-fold (from an average of 0.08). Such a decrease in P1vir burst size might be due to competition between λ and P1. Alternatively, the decrease could be due to the γ function of λ interfering with *recBC* nuclease (8), which may be required for replication of P1 because, in three-factor crosses, Hertman and Scott (11) found that burst sizes of P1 amber mutants were about 10-fold lower in *recB* than in *recB*⁺ bacteria. We do not yet know whether any significance can be attached to the finding that there is no such decrease with mutants from cluster X-2.

Results from all of the above experiments using artificial cell lysis suggest that the mutations in cluster III are in a late gene determining a lysis function and that the mutations in cluster X-2 may be in an early gene. However, these methods of lysis were unsatisfactory because the greatest increase in burst size for the mutants from cluster III was only 36-fold (to 5 PFU/cell;

TABLE 2. Burst sizes of amber mutants and P1vir grown on *E. coli* K-12 *sup*⁰ strains R594 and MF380^a

Phage	Linkage cluster ^b of amber mutation	Burst size ^c of phage grown on:		Increase in burst size (fold) ^d
		R594	MF380	
20	III	0.12	3.1	26
23	III	0.13	4.7	36
134	III	0.13	1.7	13
144	III	0.18	4.4	24
136	X-1	0.50	0.08	-6
70	X-2	0.04	0.10	3
104	X-2	0.08	0.13	2
178	X-2	0.13	0.11	1
P1vir		100	8.6 ^e	-12

^a R594(λ redA329cI857Aam32).

^b See Fig. 1.

^c Burst size was normalized to that of P1vir, set at 100. Actual burst sizes for P1vir were 75 and 93 in different experiments.

^d When phage were grown on MF380.

^e Average of two experiments (burst sizes, 7.50 and 9.64).

for *am23*, Table 2). Therefore, we tested the 15 lysis-defective mutants for their ability to produce PFU after artificial lysis of phage-infected R594 cells by lysis from without, using phage T6 (5). Mutants from linkage clusters IV, VIII, and X-1, which lyse *sup*⁰ cells without addition of T6, were included as controls. In these experiments, revertant levels among the total progeny relative to burst sizes were 10⁻⁴ to 10⁻⁶, and those for each mutant were the same whether the cells were lysed by T6 or not, indicating that any increase in burst size was due to production of amber mutants. Although the burst sizes varied in different experiments, a 38- to 992-fold increase in burst size (Table 3) was obtained with mutants from linkage cluster III, whereas an increase was not observed with *P1vir* (data not shown), with mutants from linkage cluster X-2, or with mutants from linkage clusters IV, VIII, and X-1. From these results, we conclude that the lysis deficiency of the mutants in linkage cluster III is due to a defect in a late gene determining a lysis function and that the lysis deficiency of the mutants in linkage cluster X-2 is probably due to an early gene defect.

Increase of phage titer with time. With cells infected with λ S amber mutants, phage particle production continued to increase (up to 500 to 1,000 particles per cell) after lysis would normally occur. No such increase was seen with cells infected with λ R amber mutants.

To determine, for the mutants from linkage cluster III, whether phage production continues after the normal cell lysis time for *P1vir*, samples of *am134*-infected DW101 cells were assayed for PFU at 60, 120, 180, and 240 min after infection, after lysis of the cells by T6. λ cI857*Ram*5, λ cI857*Sam*7, and *P1vir* were included as controls (Table 4). As expected, with λ cI857*Ram*5 there was no significant increase in phage production with time, but with λ cI857*Sam*7 the burst size increased to 445. With *P1vir* the burst size doubled between 60 and 120 min after infection (the reason for this is unknown) and then remained about the same between 120 and 240 min. Although the burst sizes for *am134* were low (20 to 32) in this experiment, there was no significant increase with time. We conclude that, in this respect, *am134* resembles the R mutants rather than the S mutants of λ .

Thin sections of phage-infected cells. As stated above, DW101 cells infected with mutants from linkage cluster X-2 do not produce viable phage after artificial lysis by T6, whereas cells infected with mutants from cluster III do. To see whether or not they produce any visible phage structures, thin sections of DW101 cells infected with mutants from linkage cluster X-2 were prepared at 110 min postinfection and ex-

TABLE 3. Burst sizes of amber mutants grown in DW101 *sup*⁰ cells, with and without T6 phage added for lysis

Amber mutant	Linkage cluster ^a	Burst size ^b		Increase in burst size when T6 added (fold)
		No T6	T6 added	
20	III	0.07 (0.19)	23 (21)	329 (111)
23	III	0.48 (0.29)	38 (32)	79 (110)
73	III	0.43	67	156
134	III	0.12 (0.55)	119 (47)	992 (85)
144	III	0.69 (1.5)	26 (44)	38 (29)
33	IV	0.03	0.02	— ^c
35	IV	0.02	0.02	—
56	IV	0.12	0.17	—
139	VIII	0.02	0.02	—
136	X-1	7.0 (1.1)	6.0 (0.71)	—
1	X-2	0.03	0.02	—
11	X-2	0.003	0.003	—
70	X-2	0.28	0.37	—
111	X-2	0.10 (0.08)	0.09 (0.09)	—
36	X-2	0.002	0.002	—
104	X-2	0.24	0.23	—
105	X-2	9.6 (0.02)	17 (0.02)	—
137	X-2	0.04	0.05	—
175	X-2	0.03	0.04	—
178	X-2	0.27 (0.58)	0.16 (0.49)	—

^a See Fig. 1.

^b Normalized to *P1vir* = 100. Actual burst sizes for *P1vir* were 50 to 160 in different experiments. Figures in parentheses denote results of duplicate experiments.

^c Increase in burst size was not significantly different from 1 $\times$.

TABLE 4. Burst sizes of *P1* and λ phage at various times after infection following lysis of phage-infected cells with T6

Infecting phage	Burst size after addition of T6 at post infection times of:			
	60 min	120 min	180 min	240 min
λ cI857 <i>Ram</i> 5	89	127	113	109
λ cI857 <i>Sam</i> 7	92	277	390	445
<i>P1vir</i>	49	98	109	109
<i>P1viram134</i>	23	26	32	20

amined by electron microscopy. As controls, uninfected cells and cells infected with *P1vir* or mutants from linkage cluster III were sampled at various times after infection as described below. Two techniques, differing in methods of fixation and embedding of the samples, were used to help eliminate artifacts due to these procedures. For ease of nomenclature these different techniques will be referred to as method 1 and method 2 (see Materials and Methods for details).

When method 1 was used (Fig. 5), *P1vir*-infected cells (Fig. 5A) sectioned at 55 min (i.e., just before lysis commenced) contained clearly visible phage heads, most of which appeared to

FIG. 5. Thin sections of phage-infected or uninfected DW101 (*sup*⁰) cells prepared by method 1 (see the text for details). (a) P1vir-infected cells at 55 min postinfection; (b) *am20*-infected cells at 110 min; (c) *am1*-infected cells at 110 min; (d) uninfected cells at 110 min. Bar, 1 μ m.

contain DNA as evidenced by their electron-dense appearance. Tails were rarely seen because they were smaller in diameter (18 nm) than the estimated section thickness (30 nm).

Cells infected with *am20* (linkage cluster III) and sectioned at 110 min had a similar appearance (Fig. 5B) to *P1vir*-infected cells sectioned at 55 min in having phage heads (some of which have tails) in approximately the same numbers. This was as expected because *am20*-infected DW101 cells gave an increase in burst size from 0.07 to 23 (Table 3) when they were lysed artificially by T6, and *am134*, another mutant in linkage cluster III which behaves similarly, did not give a further increase in burst size with time (Table 4).

Cells infected with *am1* (linkage cluster X-2) and sectioned at 110 min rarely contained phage heads (Fig. 5C). About 1 in 200 cell sections contained one or two heads, which could be due to leakiness of the mutant (Fig. 5C was purposely selected to show these relatively rare particles). Cells infected with *am1* contained electron-transparent areas of DNA similar to those seen in cells infected with *P1vir* (Fig. 5A) or with *am20* (Fig. 5B). However, the cytoplasm of *am1*-infected cells appeared to be disrupted, i.e., the electron-dense ribosome-containing areas seen in uninfected (Fig. 5D), *vir*-infected, and *am20*-infected cells were infiltrated with electron-transparent material which could also be DNA. With *am1*-infected cells, the ribosome-free DNA-plasm, which was fairly compact and globular, may represent primarily phage DNA, whereas the host DNA may be fully dispersed, accounting for the less crowded appearance of the ribosomes in the rest of the cell. Conversely, the compact DNA-plasm may represent host DNA whose organization has been significantly altered by infection with *am1*, and the less crowded ribosome area may represent phage DNA uniformly mixed with ribosomes. Unfortunately, host and phage DNAs cannot be distinguished in electron micrographs. However, regardless of the actual chemical composition of these less crowded ribosome areas, they are noteworthy because they are specific for *am1*-infected cells.

Similar results, as regards the presence or absence of phage particles and the appearance of electron-transparent material, were obtained with *P1vir* (Fig. 6A), other mutants from linkage cluster III (*am144*; Fig. 6B) and linkage cluster X-2 (*am105*; Fig. 6C), and uninfected cells (Fig. 6D) when method 2 was used to fix and embed the cells. A difference seen was that cells prepared by method 2 showed DNA-containing heads that were more electron dense than those in cells prepared by method 1.

DISCUSSION

We have shown that of the 103 P1 amber mutants tested, 83 cause lysis of nonpermissive cells at the same time as *P1vir*. These mutations are located in all of the linkage clusters except linkage cluster III and subgroup X-2 (Fig. 1). By analogy with other phages, these mutants would be defective in late genes involved in particle morphogenesis. This has been confirmed by recent analysis of these mutants by electron microscopy (manuscript in preparation).

Five amber mutants, with mutations located in gene 2 (linkage cluster IV), lyse nonpermissive cells prematurely. Premature lysis mutants have also been found in λ (*λ clo*; 4, 21). *λ clo* is thought to be a lysis regulator which acts by inhibiting lysis until the scheduled time for termination of the latent period. The five P1 amber mutants exhibiting premature lysis may have a defect similar to that in the *λ clo* mutants. Lysates of cells infected with one of these mutants (*am31*) do not exhibit serum blocking power (manuscript in preparation); this is probably because too few phage structures are produced, by the time lysis occurs (the burst size is 0.24 PFU/cell), to be detected by this test. In support of this hypothesis, a sample of a *P1vir* lysate, diluted to give a phage titer comparable to that of the *am31* lysate, also gives a negative result. In addition, electron microscopy (manuscript in preparation) of *am31*-infected cell lysates shows very few particles (and these are structurally complete) compared to a *P1vir* lysate. In experiments measuring the incorporation of tritiated thymidine into DNA, J. R. Scott (personal communication) found that *am31* and *am56* from gene 2 show a DNA-arrest phenotype. This phenotype might really be due to the occurrence of premature lysis, that is, DNA synthesis by gene 2 mutants may be normal except that it is stopped prematurely due to premature lysis of the cell.

The five P1 amber mutants constituting linkage cluster III do not cause cell lysis but produce viable phage particles after artificial lysis of the infected cells. In addition, electron micrographs of thin sections of infected cells show phage particles similar to those seen in thin sections of *P1vir*-infected cells. We conclude that these mutants are defective in a lysis mechanism. The five mutants probably have a mutation in the same gene, located between *am108* (linkage cluster II; Fig. 1) and the *Pst*I cleavage site (Fig. 7), because (i) they have the same single defect; (ii) they do not complement each other; and (iii) their *am*⁺ alleles are rescued from a small fragment (4.2 kilobase pairs) of the P1 genome which also contains the *am*⁺ allele of *am108* (located

FIG. 6. Thin sections of phage-infected or uninfected DW101 (*sup*⁰) cells prepared by method 2 (see the text for details). (a) P1vir-infected cell at 55 min postinfection; (b) am144-infected cell at 110 min; (c) am105-infected cell at 110 min; (d) uninfected cell at 110 min. Bar, 1 μ m.

FIG. 7. Partial physical and genetic maps of phage P1 compiled from data of Walker and Walker (31), Bächli and Arber (1), Mural et al. (16), Sternberg (27), and Baumstark and Scott, personal communication). Numbers within boxes identify digestion fragments generated by restriction endonucleases BglII, EcoRI, and BamHI (1). The order of EcoRI fragments 6 and 11 is unknown. Map units are equivalent to percent of the P1 genome, which is calculated as 58.6×10^6 molecular weight (90.15 kilobase pairs) DNA, based on the sum of the sizes of the individual digestion fragments (1). The unique PstI cleavage site has been designated as map unit 20 (32) to align the physical and genetic maps of P1. Genetic markers refer to the *c1* mutation (22) and various amber mutations (22, 30). The arrows at map unit 92 indicate the starting point and direction of DNA packaging (1).

in a different gene from the lysis mutations). We have designated this gene as gene 17.

The P1 lysis gene 17 resembles the λ R gene (which codes for an endolysin) rather than the λ S gene (whose product alters the cytoplasmic membrane) in that cells infected with amber mutants representing this gene are not lysed by addition of chloroform (data not shown) and in that the one P1 mutant tested did not continue to produce phage after the end of the normal latent period. In addition, in studies of DNA synthesis, these P1 mutants resemble R mutants rather than S mutants of λ (18), because Scott (personal communication) found that cells infected with *am20* and *am23* do not continue to synthesize DNA after the time that DNA synthesis by P1vir has ceased (at approximately the time that P1vir-infected cells lyse). Such results support the finding that phage production by these P1 mutants is not continued after the normal time for cell lysis by wild-type P1. However, in a lysozyme assay similar to that of Harris et al. (10; data not shown), a lysate of P1vir, which has the wild-type allele of the P1 lysis gene, gave a negative result (similar to that obtained with a λ cI857*Ram5* lysate) where controls of λ cI857, λ cI857*Sam7*, and T4D lysates gave positive results. Therefore, it seems that the P1 lysis enzyme coded by this gene differs, perhaps in specificity, from that coded by the wild-type λ R gene or the T4 *e* gene (29). The λ Rz gene product may be a lysozyme type of protein with a specificity different from that of the λ R gene product (33), but not enough is known about the Rz gene product or the P1 lysis gene product to make a comparison.

In addition to the P1 lysis gene described here, P1 probably has a second lysis gene which could be analogous to the S gene of λ because, as

shown by Iida and Arber (12), some deletion prophages of P1 will not lyse the host cell after induction unless chloroform is added, and these strains can accumulate up to 1,000 particles per cell. These prophages are missing a region of the genome from map unit 20 to around 30 (Fig. 7), and the amber mutations in linkage cluster III are located to the left of map unit 20 (27), approximately between map units 15 and 20. In addition to this S-like gene, gene 2 (whose absence causes premature lysis) is also located within the deleted region (16). Therefore, two P1 lysis genes and a possible lysis regulator gene are located within a region spanning less than 15% of the P1 genome.

With the 10 amber mutants in linkage cluster X-2, previous results of complementation tests in liquid (31) gave inconclusive results as to whether or not these mutations are in the same gene. We have shown that these 10 mutants, which do not lyse nonpermissive cells, do not complement each other for lysis. In addition, the *am*⁺ alleles of four of these mutations (*am178*, *am1*, *am70*, and *am105*; representing each of the three groups of mutants in cluster X-2; Fig. 1) can be rescued from a 0.62-kilobase pair fragment of the P1 genome (EcoRI fragment 20, Fig. 7; 16, 27) which would be expected to code for protein of no more than 23,000 molecular weight. Heilmann et al. (10a) have recently shown (by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of P1 amber-infected minicells) that *am1*, *am11*, and *am70* are each missing a protein of 64,000 molecular weight. Therefore it seems likely that the 10 mutations in cluster X-2 are in the same gene. (We shall subsequently refer to the 10 mutations of linkage cluster X-2 as being in gene 10, a designation originally made by Scott [22] for *am1* and *am11*.) Gene 10 does not

extend much beyond the right-hand cleavage site of *EcoRI*-20 because the adjacent fragment (*EcoRI*-22) on this side is an even smaller fragment (0.35 kilobase pair) which contains the *am*⁺ allele (*am16*⁺) of a different gene. Therefore the structural part of gene 10 must extend into the adjacent fragment to the left, *EcoRI*-6/11 (the order of *EcoRI*-6 and *EcoRI*-11 is unknown); this could explain why gene 10 is not expressed from the cloned *EcoRI*-20 fragment, as found by Mural et al. (16).

Cells infected with mutants from gene 10 do not produce viable phage or show serum blocking power after artificial lysis. In addition, the majority of infected cells do not contain any particles when examined after sectioning. The cytoplasm of the sectioned cells appears to contain material that could be phage DNA. Consistent with this hypothesis is the finding by Scott (personal communication) that cells infected with *am11* (in gene 10) incorporate tritiated thymidine in the same way as *P1vir*-infected cells. It therefore appears that mutants in this gene are defective for synthesis of late proteins and that this gene may code for a Q-like gene product, similar to that in λ (6, 13, 17), which is responsible for turning on the P1 late genes, i.e., the structural genes and the lysis gene(s). Another similarity between λ Q mutants (26) and these P1 mutants is that, upon infection with these mutants (at an MOI of about 5), more than 90% of the cells are killed.

We are currently investigating, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, P1 amber mutants from each of the linkage clusters for their patterns of protein production. If gene 10 codes for a Q-like protein, we would expect that gene 10 amber mutants would be missing all of the late P1 proteins. Heilmann et al. (10a) have recently examined minicells, separately infected with three mutants from gene 10 (*am1*, *am11*, and *am70*), by polyacrylamide gel electrophoresis and found that these mutants are missing only one protein (molecular weight, 64,000), compared to *P1vir*. Our results from serum blocking power tests and from examining thin sections of cells infected with *am1* or *am105* suggest that all of the late P1 proteins are missing. Reeve (20) has found that, in minicells, T5 phage synthesizes only pre-early and some early polypeptides, and it may be that the early genes of P1 (including gene 10) are expressed in minicells but that the late genes of P1 are not.

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