New Genes in the Left Arm of the Bacteriophage $\phi 80$ Chromosome

PHILIP YOUNDERIAN[†]* AND JONATHAN KING

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

We describe the isolation and partial genetic characterization of 247 amber (suppressor-sensitive) mutants of temperate bacteriophage $\phi 80$ of *Escherichia coli*. Of these 247 mutants, the mutations of 201 mapped to the left arm of the $\phi 80$ chromosome and the mutations of 39 mapped to the right arm of the genome. Complementation tests among these and previously described left arm mutants defined five additional genes in the left arm of the chromosome. The positions of these genes are consistent with the hypothesis that four of them represent functions essential for $\phi 80$ tail assembly and one represents a capsid assembly function, probably the major coat protein. The identification of these genes brings the $\phi 80$ genome into closer correspondence with the organization of the phage lambda genome. Two- and three-factor crosses performed between mutants with defects in each of the previously identified genes and mutants with defects in the five new genes allowed us to construct a consistent, roughly additive recombination map of the left arm of the bacteriophage $\phi 80$ genome.

Temperate coliphage $\phi 80$ is a member of the lambdoid group of bacteriophages. It exhibits the four primary characters of a subgroup of this group: it is inducible by irradiation with UV light (10), it possesses 12-nucleotide-base cohesive ends (22), it may recombine in coinfections with phage λ to form a variety of viable hybrids (17), and each particle is composed of an isometric head and a long noncontractile tail (16). $\phi 80$ has been used extensively as a tool for the specialized transduction of host *Escherichia coli* K-12 markers and more recently has played a central role in the development of host-vector systems for endonuclease-assisted recombination in vitro (2).

As part of a continuing analysis of the genetic control of phage tail morphogenesis (7, 21), we have been comparing the genetic control of $\phi 80$ tail morphogenesis with the control of the well-characterized lambda tail assembly pathway (5, 6).

To gain information concerning the genetic structure of the $\phi 80$ chromosome, Sato and co-workers (15) isolated 53 suppressor-sensitive (amber) mutants of $\phi 80$, which they classified into 19 different complementation groups; the mutations of 13 of these groups are located on the left arm of the $\phi 80$ chromosome. A subsequent mutant search resulted in the identification of six additional complementation groups containing amber alleles, which have been assigned to the right arm of the genome (14). The mutations of these 19 complementation groups

have been ordered with respect to one another by mapping the amber alleles which they contain against a set of defective lysogens carrying deletions extending into $\phi 80$ prophage (15), by two-factor crosses (14, 15), and by three-factor crosses (8).

Like the genome of λ , the $\phi 80$ chromosome (see Fig. 4) consists of three regions of approximately equal size: the left arm, concerned with the morphogenetic functions of DNA encapsulation and particle assembly; the central portion, concerned with site-specific and vegetative recombination functions; and the right arm, concerned with the functions of immunity, DNA replication, and host cell lysis (14, 15). Within the left arm of the $\phi 80$ chromosome, the head genes are clustered to the left of the cistrons responsible for tail morphogenesis, as is the case for the left arm of the bacteriophage λ chromosome (12).

Initial studies in this laboratory revealed that the 13 previously defined cistrons of the $\phi 80$ genome left arm did not include several of the genes coding for the head and tail structural proteins which were identifiable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of labeled defective lysates. We wished to isolate mutants characterizing these genes for use in experiments to determine how the length of the lambdoid tail is specified. Below, we describe the identification of five additional cistrons mapping in the $\phi 80$ genome left arm and some of the genetic properties of the amber mutations defining $\phi 80$ cistrons.

A similar search has been performed inde-

[†] Present address: Department of Microbiology, University of Massachusetts Medical School, Worcester, MA 01605.

pendently by Rybchin and co-workers in Leningrad, who also found five new left arm genes (13).

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used were all derivatives of *E. coli* strain K-12. Strain 594 (*strA594*) is suppressor⁻ (*sup*⁻) and was used as the restrictive host for amber mutants (20); strains M107 (*supD*), from S. Brenner, ECO (*supE*) (17), and QD5003 (*supF*) (4) were used as the permissive hosts for plating and stock growth. All bacterial strains were obtained from the collection of E. Signer.

Bacteriophage strains. All of the $\phi 80$ strains isolated in this study were derived from the virulent, clear plaque-forming strain $\phi 80$ vir. $\phi 80$ vir was picked as a spontaneous mutant from a stock of $\phi 80$ wild type which could form plaques on a lysogen of $\phi 80$. The spontaneous appearance of virulent $\phi 80$ in a stock of wild type occurs at the frequency of a single-step mutation (Signer, unpublished data). The amber alleles numbered from 1 through 200 were isolated from $\phi 80$ wild type on strain CA5013 (*supD*) by Sato et al. (15) and were kindly provided by A. Matsushiro. The alleles numbered from 401 to 500 were isolated on *E. coli* M107 (*supD*) as the permissive indicator; the alleles numbered from 501 to 600 were isolated on strain ECO (*supE*) as the permissive indicator; and the alleles numbered from 601 to 700 were isolated on strain QD5003 (*supF*) as the permissive indicator.

Media. Tryptone broth contained (per liter of distilled water) 10 g of tryptone (Difco Laboratories) and 2.5 g of NaCl. In addition, tryptone top and bottom agars for the plating of phage and bacterial cells contained 0.7 and 1.1% agar, respectively. For experiments involving the growth of phage in liquid culture, tryptone broth was supplemented with a 1:100 dilution of 1.0% yeast extract. XM medium, which was used for the UV mutagenesis of phage, was X medium (containing, per liter of distilled water, 14.4 g of Na₂HPO₄·12H₂O, 2.5 g of KH₂PO₄, 1.5 g of KCl, and 1.0 g of NH₄Cl) made 10 mM in MgSO₄·7H₂O immediately before use. TM buffer (10 mM Tris-10 mM MgSO₄·7H₂O, titrated to pH 7.5 with concentrated HCl) was used as a diluent for bacterial and phage assays.

Preparation of rabbit anti- $\phi 80$ antiserum. Anti- $\phi 80$ antiserum was prepared by subcutaneous injection of two 2.5-kg virgin female white New Zealand rabbits with a total of 2×10^{12} PFU of $\phi 80$ wild type from a plate lysate in Freund complete adjuvant. The phage were divided equally between two injections, which were spaced 3 weeks apart. At 2 weeks after the second injection and at 3-week intervals thereafter, each of the rabbits was bled from an ear vein for 3 consecutive days, yielding approximately 50 ml of whole blood per rabbit at each bleeding. The whole blood was allowed to clot at 4°C for 24 h, and each preparation was cleared by low-speed centrifugation. The whole serum, used in all experiments, displayed a first-order rate constant (*k*) of inactivation of about 50 min⁻¹.

Plaque-forming methodology. Phage were assayed with bacteria which were grown to a logarithmic-phase concentration of 1×10^8 to 3×10^8 cells per ml at 30°C in tryptone broth with rapid aeration

supplied by bubbling and then chilled with continued aeration in an ice water bath to 4°C; 0.1 ml of a dilution of the phage to be titrated was added to 0.5 ml of plating bacteria and then incubated at room temperature (25°C) for 10 to 15 min. A 2-ml amount of melted top agar stored at 45°C was then added to these infected cell complexes, and the mixture was poured onto petri dishes containing 40 ml of solid bottom agar. After the top agar had solidified, the plates were incubated for 24 to 48 h at 30°C or room temperature.

Single plaques were isolated by inserting a sterile flat wooden toothpick into a plaque, stabbing the moistened toothpick into a plate containing 40 ml of solidified bottom agar, and overlaying the plate with a mixture containing 0.5 ml of permissive plating bacteria and 2.0 ml of melted top agar. After 24 h of incubation at 30°C, plaques prepared in this manner exhibited large regions of clearing in the area of the stab, as well as isolated single plaques radiating out from the center of the stab.

Phage crosses and liquid complementation procedure. A chilled exponential culture of *E. coli* ECO (for phage crosses) or of strain 594 (for liquid culture complementation tests) was diluted 1:50 into tryptone broth at 30°C and grown with rapid aeration provided by bubbling to an exponential-phase density of 1×10^8 to 3×10^8 cells per ml. The bacteria were then chilled in an ice bath to 4°C, pelleted by low-speed centrifugation, and suspended in cold (4°C) TM buffer to a final concentration of 10^8 cells per ml. A mixture of the two parental phages, each at a concentration of 10^{10} phage per ml, was added to an equal volume of the suspended cells to yield a final concentration of 5×10^8 cells per ml and a multiplicity of infection of each input phage type of 10. The infection mixture was then incubated at 4°C for 2 to 4 h, after which less than 20% of the input phage remained as free phage in suspension; 1 to 2 drops (0.05 to 0.10 ml) of anti- $\phi 80$ antiserum (*k* = 1.0 min⁻¹) was added, and the mixture was then allowed to incubate at room temperature without aeration for 15 min to decrease the number of remaining free phage. The infective centers were then diluted 10^4 into prewarmed (30°C) tryptone broth and incubated at 30°C for 3 h. Several drops of chloroform were then added to each infection mixture, and the mixtures were blended gently with a Vortex mixer and then assayed for plaque-forming units on the various indicators.

Complementation spot tests. Spot tests were performed by seeding a plate with strain 594 to which 0.1 ml of an amber mutant tester stock at a titer of 10^8 PFU/ml had been added. The plates were then spotted with approximately 10 μ l containing 10^5 to 10^6 PFU of the amber mutants to be tested against the seeded mutant. The plates were incubated overnight at either 30°C or room temperature (25°C). Spots which produced a confluent area of lysis were taken as an indication that the two amber mutants tested were defective in different genes. Recombination between pairs of amber mutants defective in the same gene to produce am⁺ progeny did not yield a confluent area of lysis under these conditions, but rather formed small numbers of tiny plaques within the area of the spots.

Isolation and characterization of phage mutants. A 1:50 dilution of a chilled exponential culture

of either M107, ECO, or QD5003 was grown in tryptone broth at 30°C to a concentration of 2×10^6 cells per ml; 25 ml of the culture was then pelleted by low-speed centrifugation, and the pellet was suspended in 5 ml of TM buffer and irradiated at a final dose of 300 ergs/mm² with a General Electric G15T8 15-W germicidal UV lamp.

New amber mutants of $\phi 80_{vir}$ were picked from a UV-mutagenized stock by double-layer mixed indicator screening (12). A dilution of a $\phi 80_{vir}$ stock that had received a UV dose of 1.6×10^3 ergs/mm² was mixed with 0.2 ml of the UV-irradiated indicator (about 2×10^6 original cells), which was sufficient to produce about 300 plaques on the irradiated host. After 10 min of incubation at room temperature, 2.0 ml of melted top agar was added to this mixture, and the mixture was then plated and allowed to harden. After an additional 10 min, 0.5 ml of a 1:100 dilution of a saturated stationary-phase culture of strain 594 in tryptone broth (about 2×10^7 cells) and 2.0 ml of melted top agar were mixed and layered on top of each plate.

The plates were then incubated for 48 h at 30°C for the indicators ECO (*supE*) and QD5003 (*supF*) and for 48 h at 36°C for the indicator M107 (*supD*). Approximately 1,000 uniformly turbid plaques from these mixed indicator plates were chosen as putative amber mutants on each of the three permissive strains; these plaques were then stabbed with a sterile round toothpick into lawns of strain 594 and their respective suppressing hosts. These turbid plaques comprised about 0.6% of the total number of plaques screened. Approximately 100 of the phage strains, which cleared larger areas around the stabs on lawns of their suppressing hosts than on lawns of the nonpermissive strain 594, were chosen as amber candidates on each of the three permissive hosts. Single plaques of the turbid candidates were then isolated on their respective permissive strains and retested for the inability to grow on strain 594 after stabbing.

The retested stabs which grew on the permissive indicators but not on strain 594 were then removed with toothpicks from the permissive lawns, placed into buffer, and replated on permissive lawns to reisolate single plaques, which were then suspended in 1.0 ml of TM buffer. From these suspensions, working stocks were grown by the plate lysis method (1).

RESULTS

Isolation and characterization of new amber mutants. Two separate stocks of $\phi 80_{vir}$ were irradiated with UV light, plated onto double-layer mixed indicator plates, and screened for amber mutants as described above. A total of 263 independent amber mutants of $\phi 80$ were isolated on the three permissive strains. From the first stock, which had a survival titer of 0.1% when plated on UV-irradiated *E. coli* M107 (*supC*), the series of 95 amber mutants numbered from 401 through 500 were isolated from a total of 51,500 plaques. From the second stock, which had a survival titer of 0.2% when plated on UV-irradiated *E. coli* ECO (*supD*) or QD5003

(*supF*), the series of 88 amber mutants numbered from 501 through 600 were isolated on *E. coli* ECO from a total of 72,100 plaques, and the series of 80 amber mutants numbered from 601 through 700 were isolated on *E. coli* QD5003 from a total of 138,000 plaques.

The survival titers of the mutagenized $\phi 80_{vir}$ stocks on the UV-irradiated indicators were approximately 100- to 200-fold greater than the survival titers of the stocks on indicators which had not been irradiated; this was similar to the maximum amount of UV reactivation observed for phage λ (19).

Suspensions of single plaques of the isolates were diluted appropriately and plated onto permissive and restrictive hosts. The ratio of the number of plaque-forming units on the restrictive indicator *E. coli* 594 to the number of plaque-forming units on the permissive indicator was taken as a measure of reversion frequency. A total of 16 of the amber mutants isolated by our procedure were found to have reversion frequencies of 10^{-4} or greater and were excluded from further characterization, leaving 247 to characterize. We found no amber mutants which had reversion frequencies of less than 10^{-8} . We took this result as an indication that few of our isolates were double amber mutants.

To determine its complementation group, each of the 247 new amber isolates was spot-tested against one reference amber mutant defective in each of the previously identified morphogenetic genes (genes 1 through 13) (15). A total of 135 of the amber mutants were unambiguously assigned to one of the complementation groups 1 through 13 by these spot tests as described above. Drops of these mutants showed confluent clearing on lawns seeded with representative amber mutants in genes 1 through 13 with the exception of one tester amber mutant. Drops of a mutant on a lawn of strain 594 seeded with the exceptional tester amber mutant were indistinguishable from spots on strain 594 alone; both exhibited no visible clearing of the lawn.

Many of the other amber mutants gave ambiguous results in these spot tests. Spots of new isolates containing amber mutations corresponding to genes 8 and 10 on lawns seeded with 8⁻ and 10⁻ amber tester phages were particularly difficult to interpret; they were often indistinguishable in their degrees of clearing. Several amber isolates containing mutations eventually shown to belong to gene 11 were initially complemented into gene 7 and a new complementation group immediately to the right of gene 7 on the genetic map (see below). Careful replication of many of the spot tests (sometimes involving the comparison of spots of serial two-fold dilutions of a suspension of an amber phage

plaque) allowed us to confidently assign 24 additional alleles to complementation groups in genes 1 through 13.

In addition, 24 of the amber mutants were unable to complement any of the previously identified cistrons of the left arm of the $\phi 80$ chromosome. These complementation spot tests were originally performed with the tester amber phages in a *vir*⁺ genetic background. When the tests were repeated with amber representatives of cistrons 1 through 13 also carrying the *vir* allele, the 24 amber phages were able to clear the area of a spot. These 24 amber phages were also unable to complement mutants with mutations in the right arm of the phage chromosome also carrying the *vir*⁺ allele or to clear a spot on a lawn of a nonpermissive $\phi 80$ wild-type lysogen. The mutations in these mutants probably affect genes involved in the control of lysogeny and immunity. When spot-tested against one another, these mutants defined two complementation groups, groups I and II.

The remaining 64 isolates with amber mutations which were not in any of the known morphogenetic cistrons were then spot-tested against each other. These spot tests defined nine additional complementation groups. Thus, in addition to the morphogenetic genes, a total of 11 cistrons were included among the new amber phages. These mutants presumably included isolates which contained mutations that were representatives of the early $\phi 80$ genes, since the new isolates had not been complemented against existing mutants with mutations mapping in the right arm of the $\phi 80$ chromosome.

Before further complementation analysis, we assigned representatives of these complementation groups to one of the arms of the $\phi 80$ chromosome. To position the new complementation groups on the genetic map of $\phi 80$, we first performed backcrosses between representatives of the new groups (amber *vir* isolates) and $\phi 80$ wild type to obtain amber *vir*⁺ recombinants. Amber mutants in five of the new complementation groups (groups I through V) yielded such recombinants at a low frequency of recombination (<0.5%), whereas mutants in the other six groups (groups VI through XI) yielded such recombinants at a frequency of recombination of 2.0% or higher. Since these recombination frequencies were a measure of the linkage of the amber allele with the *vir* allele, it appeared likely that the alleles of groups I through V, which were more tightly linked with *vir*, would map in the right arm of the phage, whereas the alleles of groups VI through XI, which were less tightly linked with *vir*, would map in the left arm. The *vir* allele had been mapped previously to the immunity region of $\phi 80$ by Rybchin and Signer

(personal communication).

The alleles of representatives of several groups (groups VII, IX, X, and XI) were then directly mapped by three-factor crosses (see below) to positions in the left arm of the $\phi 80$ chromosome. These amber representatives were shown first to be tightly linked with amber alleles in the left arm and then were positioned between two previously identified cistrons of the left arm.

Representatives of the remaining new complementation groups were then crossed with several tester amber phages. The results of these crosses are shown in Table 1. Mutants in the five groups with mutations more tightly linked with the *vir* allele in backcrosses recombined to produce am⁺ progeny at much lower frequencies of recombination with the right arm mutant than with the left arm mutants. The frequencies of recombination observed in crosses involving mutants in groups I, II, and III were generally lower than the frequencies of recombination in those crosses involving representatives of the other new groups. Representatives of groups VI and VIII recombined at lower frequencies with left arm amber phages than with the right arm amber phage. Mutants with mutations mapping in the right arm of the $\phi 80$ genome by these two tests were not considered further, since we were primarily interested in those mutations affecting phage morphogenesis.

Genetic map of the left arm of the $\phi 80$ chromosome. To position the new amber mutations on the genetic map of $\phi 80$, we first back-

TABLE 1. Positioning of new complementation groups^a

Group	Allele	Recombination distances (%) for the following tester amber alleles:			
		Left arm		Right arm	
		6 ⁻ am21	am417	10 ⁻ am14	18 ⁻ am91
I	am501	3.0	2.6	1.2	0.8
II	am504	4.6	1.8	2.6	2.2
III	am562	2.8	2.2	4.1	0.4
IV	am536	10.2	14.2	5.4	1.0
V	am636	7.6	7.6	5.6	2.8
VI	am579	0.2	1.2	3.6	10.4
	am557	0.8	2.0	4.6	9.4
VIII	am591	0.4	1.0	5.6	6.8

^a Crosses were performed between the combinations of alleles listed in the table according to the procedures described in the text. Both alleles were present in these crosses in a *vir* background. The values are recombination distances (percentages) between the two amber alleles, calculated as twice the titer of the progeny of a cross on strain 594 (am⁺ recombinants) divided by the titer of the progeny on strain ECO (total progeny). Two amber representatives of group VI are shown to illustrate that these results are not allele specific for the representatives of a particular complementation group. am417 is a representative of complementation group VIII.

crossed representatives of each of the new complementation groups with mutations mapping in the left arm (amber *vir* isolates) with $\phi 80$ wild type to obtain amber *vir*⁺ recombinants. This also served to reduce the number of secondary mutations introduced during the mutagenesis of $\phi 80$ *vir*, by outcrossing the amber alleles from the mutagenized background. To conduct reciprocal crosses, mutants with the amber alleles from the series 1 through 200, representing each of the previously identified morphogenetic cistrons, were crossed from their *vir*⁺ background with $\phi 80$ *vir* to obtain amber *vir* recombinants.

The reversion frequencies of the amber mutants used in the genetic analyses ranged between 10^{-7} and 10^{-5} for all alleles. The nonpermissive burst sizes of all of the amber mutants used in the genetic analyses after a single round of growth on strain 594 were less than 2 PFU/cell.

Our mapping scheme employed *vir* as an outside marker in crosses of the type $\phi 80$ amA *vir* $\times$ $\phi 80$ amB *vir*⁺ in order to orient two markers with respect to one another and the immunity region; mapping was accomplished by reciprocal crosses of the form $\phi 80$ amA *vir*⁺ $\times$ $\phi 80$ amB *vir* and $\phi 80$ amA *vir* $\times$ $\phi 80$ amB *vir*⁺ (Fig. 1). The half-recombination frequency for each cross was determined by dividing the titer of am⁺ progeny (number of plaque-forming units on strain 594) by the number of total progeny (number of plaque-forming units on strain ECO). In addition, we determined the percentages of turbid (*vir*⁺) and clear (*vir*) plaque-forming progeny among the total am⁺ progeny of the crosses. As Fig. 1 shows, the formation of am⁺ *vir*⁺ recombinants required at least a single crossover event

in one of the reciprocal crosses (Fig. 1a) and at least a double crossover event in the other (Fig. 1b). Since single crossover events should be more frequent than double crossover events, the correct order of the amA and amB alleles with respect to the *vir* marker was determined by noting which of the reciprocal crosses yielded a larger percentage of the turbid plaque-forming (*vir*⁺) am⁺ recombinants.

Although we found that phage carrying the *vir* marker were partially dominant over phage carrying the corresponding wild-type allele, resulting in a ratio of *vir* to *vir*⁺ of about 3:1 in the output progeny of a mixed infection with equal input multiplicities of the two parental types, a comparison of the percentages of am⁺ *vir*⁺ progeny of the total am⁺ progeny of reciprocal crosses provided a unique, reproducible ordering of the amber alleles with respect to one another and the *vir* immunity marker.

A summary of these results is presented in Fig. 2. The new complementation groups were given gene numbers as follows: group VI, gene 20; group VII, gene 21; group VIII, gene 22; group IX, gene 23; group X, gene 24. These assignments are in accordance with the terminology proposed by Sato et al. (15).

To evaluate the effects of negative interference on the additivity of two-factor recombination distances, the results of a number of the reciprocal crosses were analyzed (Fig. 3). The data indicate that the two-factor distances were roughly additive from approximately 0.5 to 5.0% recombination. Although negative interference did not appreciably affect the additivity of the recombination distances derived from the two-factor crosses, as is also the case for amber mutants in the left arm of the phage λ chromosome, the departure from additivity for the two-factor distances in the left arm of the $\phi 80$ chromosome was about twice that observed for two-factor distances in the left arm of the λ chromosome (12). Since the construction of a mapping function was unnecessary, the map of Fig. 4 was obtained by directly converting recombination frequencies into relative distances.

Liquid culture complementation tests. To confirm the original spot complementation results with the amber mutants defining new complementation groups, complementation tests were performed in liquid culture. In these tests, nonpermissive bacteria were infected with equal multiplicities of the two amber mutants to be tested, and the total phage yield produced at the end of one lytic cycle of growth was measured. Nonpermissive cells (strain 594) infected with two different parental phages carrying amber mutations in the same gene typically produced less than 2 PFU/infected center. Two amber

FIG. 1. Mapping $\phi 80$ amber alleles. Reciprocal crosses were performed between amA *vir* and amB parents and between amA and amB *vir* parents. The frequency of am⁺ *vir*⁺ progeny indicated the order of the two mutations.

FIG. 3. Additivity of recombination distances. The frequency of recombination (R) was calculated as described in the text. The frequency of recombination between sites a and c (R_{ac}) was plotted as a function of the sum of the primary intervals R_{ab} and R_{bc} (18). The data were taken from three separate experiments (marked by different symbols). Burst sizes in these crosses ranged from 50 to 500 phage per cell.

mutants which are able to complement one another commonly yield from 20 to 200 PFU/infected center, indicating that the mutants are defective in different functions and, therefore, different genes.

For each complementation group tested, a number of amber alleles of that gene were complemented with two representative amber alleles in the same gene and with one representative amber allele in each of the two pairs of cistrons flanking the gene of interest, as determined by the map order shown in Fig. 4. Since the left arm of the $\phi 80$ genome is transcribed from left to right (9), the observation of relatively low complementation between adjacent genes may reflect the polar effects of an amber mutation on the expression of the gene or genes to the right of the gene in which it maps. From the burst size data shown in Tables 2 through 7, it is clear that alleles in gene 20 can complement 3⁻ and 4⁻ amber alleles as well as 5⁻ and 6⁻ amber alleles but not amber alleles in the same gene. The mutant 20⁻ am554, although adsorbing as efficiently as wild type, complemented amber mutants having mutations in adjacent genes much more poorly than the other 20⁻ mutants.

Similarly, 21⁻ alleles complemented 5⁻, 6⁻, 22⁻, and 7⁻ amber alleles but not one another.

22⁻ amber alleles clearly complemented amber mutations in cistrons 6, 21, and 23. One of

the alleles (am547) complemented only poorly, and all of the 22⁻ amber alleles yielded low burst sizes when grown nonpermissively with 7⁻ amber alleles.

23⁻ amber alleles complemented amber alleles in genes 22, 7, 9, and 10; 7⁻ amber alleles were complemented poorly by 23⁻ amber alleles.

24⁻ amber alleles complemented 11⁻, 12⁻, and 13⁻ amber alleles.

Three amber alleles, am403, am445, and am645, were originally classified in complementation group XI by spot complementation tests. The representative of this group, am445, was shown to map between 9⁻ am4 and 10⁻ am14 by three-factor crosses (data not shown). However, liquid complementation tests showed that amber mutations in gene 10 and group XI are in the same complementation group. As Table 7 shows, mutations in class XI do not complement 10⁻ amber mutations; however, 10⁻ am14 complements amber mutations in the adjacent cistrons (23, 9 and 8) only poorly, as do the class XI alleles.

The poor complementation observed between amber mutations in cistrons 7 and 23 was possibly due to the polar effects of 7⁻ amber mutations. Since, in mixedly infected cells, phage carrying the *vir* allele are partially dominant over phage carrying the *vir*⁺ allele, one method to determine whether amber mutations in one gene are strongly polar on the expression of another gene is to compare the yields of reciprocal mixed *vir* and *vir*⁺ complementations between the alleles in question. In fact, 7⁻ am *vir* × 23⁻ am *vir*⁺ complementations yielded about twice the number of progeny as the reciprocal 7⁻ am *vir*⁺ × 23⁻ am *vir* complementations. This is consistent with the hypothesis that 7⁻ amber mutations are polar upon the expression of the product of gene 23. The low complementation observed between 22⁻ and 7⁻ alleles was most probably not due to polar effects; reciprocal complementations between amber mutations in these genes yielded similar numbers of progeny. Although we did not examine alleles in genes 8 and 10 by this method, we suspect that mutations in these genes may exhibit polar effects on the expression of other phage $\phi 80$ left arm genes, since alleles in these genes complement alleles in adjacent genes only very poorly.

Combined with the mapping data shown in Fig. 2, these complementation results suggest that the amber alleles in complementation groups 20, 21, 22, 23, and 24 define five new genes in the left arm of the $\phi 80$ chromosome.

DISCUSSION

Genetic map. Table 8 contains a summary of the new amber mutants obtained in this study,

FIG. 4. Genetic maps of bacteriophages λ and $\phi 80$. The percent scale of molecular distance (a) is presented in terms of the length of the lambda genome, which was taken to be 100%. The genomes of λ (b) and $\phi 80$ (c) are represented as solid and dashed lines, respectively. The positions of selected genes on the λ chromosome are according to Fianadt et al. (3). The positions of genes on the $\phi 80$ chromosome are from Sato et al. (14, 15), this work, and Youderian and King (manuscript in preparation). λ and $\phi 80$ genes are clustered in functional groups, some of which are indicated in the center of the figure. The length of each double-stranded DNA is shown in kilobases (d), assuming that 1% of the lambda genome is 492 base pairs long (Blattner, personal communication).

TABLE 2. Liquid culture complementation tests: group VI

Group VI allele	Burst size (no. of phage per cell) with:					
	Selves	3 ⁻ am6	4 ⁻ am45	20 ⁻ am579	20 ⁻ am557	5 ⁻ am9
20 ⁻ am579	0.29	140	98	0.29	0.75	120
20 ⁻ am554	0.46	8	9	0.46	0.55	18
20 ⁻ am557	0.14	180	96	0.73	0.14	150
20 ⁻ am573	0.12	97	100	0.64	0.27	92

TABLE 3. Liquid culture complementation tests: group VII

Group VII allele	Burst size (no. of phage per cell) with:					
	Selves	5 ⁻ am9	6 ⁻ am21	21 ⁻ am441	21 ⁻ am450	22 ⁻ am417
21 ⁻ am441	0.60	51	24	0.60	1.4	38
21 ⁻ am450	0.40	44	47	2.2	0.40	55
21 ⁻ am572	1.0	33	38	2.0	1.6	68
21 ⁻ am574	0.20	79	32	0.32	0.46	85

TABLE 4. Liquid culture complementation tests: group VIII

Group VIII allele	Burst size (no. of phage per cell) with:					
	Selves	6 ⁻ am21	21 ⁻ am441	22 ⁻ am417	22 ⁻ am547	7 ⁻ am11
22 ⁻ am417	0.35	11	93	0.35	0.77	19
22 ⁻ am547	1.2	5.0	5.2	1.0	1.2	3.1
22 ⁻ am591	0.30	17	26	1.7	1.4	6.8
22 ⁻ am602	0.53	31	26	0.24	0.73	8.0

TABLE 5. *Liquid culture complementation tests: group IX*

Group IX allele	Burst size (no. of phage per cell) with:					
	Selves	23 ⁻ am417	7 ⁻ am11	23 ⁻ am493	23 ⁻ am534	9 ⁻ am4
23 ⁻ am493	0.23	53	25	0.23	0.73	46
23 ⁻ am534	0.51	34	4.8	0.73	0.51	26
23 ⁻ am608	0.60	29	27	0.44	0.61	69

TABLE 6. *Liquid culture complementation tests: group X*

Group X allele	Burst size (no. of phage per cell) with:					
	Selves	11 ⁻ am7	12 ⁻ am39	24 ⁻ am453	24 ⁻ am657	13 ⁻ am28
24 ⁻ am453	0.58	79	35	0.58	1.2	69
24 ⁻ am498	ND ^a	99	86	0.61	0.86	62
24 ⁻ am623	0.77	194	26	0.54	0.66	110
24 ⁻ am657	1.2	81	61	1.2	1.2	81

^a ND, Not determined.TABLE 7. *Liquid culture complementation tests: group XI*

Group XI allele	Burst size (no. of phage per cell) with:						
	Selves	23 ⁻ 493	9 ⁻ am4	am403	am645	10 ⁻ am14	8 ⁻ am2
am403	0.69	11	10	0.69	0.51	0.60	19
am445	0.29	29	16	0.56	0.57	1.8	9.2
am645	2.2	21	20	0.51	2.2	2.2	11
10 ⁻ am14	0.30	59	12	0.60	1.8	0.30	12

classified by genes. No new amber mutations were found in the previously characterized cistrons 6 and 12. Mutants with amber mutations in at least five additional new genes were isolated; these new genes have been assigned the numbers 20 through 24, in accordance with the nomenclature proposed by Sato et al. (15). Of the 247 isolates examined in this study, the mutations of at least 201 were found to map in the left arm of the $\phi 80$ chromosome and the mutations of at least 39 were found to map in the right arm of the genome. The right arm mutants have not been characterized further.

Figure 4 summarizes the genetic maps of bacteriophages lambda and $\phi 80$, showing the locations of the five additional cistrons. By analogy with lambda, four of these cistrons specify proteins required for tail assembly, and one (gene 20) functions in capsid assembly. Our mapping of genes 23 and 24 corresponds to the results found by Rybchin et al. (13) by deletion mapping. There is a discrepancy with gene 22; our order is 6-21-22-7, and their order is 6-21-7-22. Both of these results were based on three-factor crosses. The gene 20 identified by Rybchin et al. was defined by temperature-sensitive mutants and mapped between genes 3 and 4. Subsequently, they identified two additional genes, genes 25 and 26 (personal communication), one of which maps between genes 4 and 5. The relationship between our gene 20 shown in Fig.

4 and their genes 20, 25, and 26 needs to be clarified. We have sent our mutants to Leningrad to resolve these ambiguities.

The order of the genes in the left arm of the $\phi 80$ chromosome derived from the results of this study (Fig. 1 and 2) is inconsistent with the order of genes determined by a previous isolation and characterization of mutants (15). In the previous study, gene 8 was positioned between genes 7 and 9; our results indicate that the proper order of these genes is 7-(23)-9-10-8-11 (Fig. 3). The order of genes determined in this study is consistent with the results obtained by mapping the mutations of previously isolated amber mutants against various prophage deletions carried in defective lysogens (15). Since alleles in genes 9, 10, and 8 were not separated by deletion endpoints in the previous study, we attribute the mismapping of gene 8 to the inconclusiveness of the two-factor crosses used in determining its map position, in keeping with the suggestion of Sato and co-workers (15). Rybchin et al. (13) reported the same correction.

Gene function. We have studied the proteins present in purified phage particles by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Youderian and King, manuscript in preparation). The genes coding for two of the three major structural proteins of the mature virion have not been identified yet. We isolated no amber mutants with mutations in the genes

TABLE 8. Assignment of new mutants to complementation groups

Cistron	No. of isolates	No. of sites ^a	Alleles ^b
1	12	≥ 1	3 ⁺ , 440, 449, 454, 468, 484, 500, 503, 506, 511, 516, 540, 576
2	18	≥ 5	<u>1</u> , 412, 414, 418, 436, <u>437</u> , 465, <u>489</u> , 495, 496, 531, <u>580</u> , 620, 630, 655, <u>659</u> , 679, 690
3	9	≥ 4	6, 450, 463, 527, 569, 631, 643, 661, 669, 694
4	10	≥ 2	<u>45</u> , 402, 406, 421, 438, <u>490</u> , 508, 522, 533, <u>581</u> , 650
20	9	≥ 2	<u>579</u> , 404, 475, 554, <u>557</u> , 573, 614, 668, 681
5	3	≥ 1	<u>9</u> , 505, 587, 615
6	None	1	<u>21</u>
21	9	≥ 1	<u>441</u> , 450, 472, 474, 537, 565, 572, 574, 578
22	13	≥ 3	<u>417</u> , 405, 444, 480, 485, 547, 591, 602, 612, 629, 664, 678, 693
7	42	≥ 4	<u>11</u> , 48, 410, 411, 416, 419, <u>422</u> , <u>423</u> , 424, 426, 425, 446, 447, 456, 457, 461, 462, 471, 477, 479, 481, 512, 514, 525, 541, 548, 556, 601, 617, 618, 622, 625, 626, 634, 638, 640, 641, <u>642</u> , 652, 665, 672, 677
23	3	≥ 2	<u>493</u> , 534, 608
9	13	≥ 1	<u>4</u> , 409, 413, 497, 507, 523, 529, 530, 583, 585, 586, 589, 606, 637
10	6	≥ 2	<u>14</u> , 403, 443, 535, 538, 645
8	5	≥ 2	<u>2</u> , 448, 632, 651, 673, 689
11	36	4	<u>7</u> , 26, 29, 408, 420, 425, 429, 431, 439, 482, 483, 486, 518, 539, 542, 545, 549, 550, 553, 558, 568, 582, 584, 588, 605, 609, 610, <u>615</u> , 619, 621, 624, 635, 644, 647, 648, 671, 675, 680
12	None	1	<u>12</u>
24	6	≥ 1	<u>453</u> , 455, 498, 623, 657, 676
13	6	≥ 4	<u>28</u> , <u>451</u> , <u>467</u> , 524, 627, 660, 684

^a The number of sites within each gene was calculated as more than or equal to the smallest number of alleles having uniquely different patterns of suppression plus the number of those alleles sharing the same suppression pattern which were demonstrated to recombine with one another to produce am^+ progeny at a frequency greater than background reversion.

^b The genes are listed in the order in which they occur on the genetic map; the first allele listed for each complementation class is the representative amber allele for that class, which was used to construct the genetic map of the left arm of the genome. Mutants 1 through 53 were obtained from A. Matsushiro; these were not included in the number of isolates but were included in the number of sites column. The alleles defining sites in the same gene known to be distinct are underlined.

coding for the major structural protein of the tail (28,000 molecular weight) and the second-most-abundant component of the capsid (11,700 molecular weight).

One of the new cistrons, gene 20, codes for the major structural protein of the phage capsid (Youdarian and King, manuscript in preparation), coded by gene E in phage lambda. Thus far, six genes (1, 2, 3, 4, 20, and 5) required for the formation of the $\phi 80$ phage capsid have been identified (15; unpublished data). Bacteriophage λ , whose capsid composition closely resembles that of $\phi 80$, requires the action of at least 10 phage gene products for capsid morphogenesis (11). Therefore, it is likely that not all of the $\phi 80$ head genes have yet been identified; one of these unidentified genes corresponds to the second-most-abundant component of the phage capsid (Youdarian and King, manuscript in preparation).

The λ tail requires the action of at least 11 identified phage genes for its morphogenesis (6). Eleven genes (6, 21, 22, 7, 9, 23, 10, 8, 11, 24, and 13) which are required for the formation of the $\phi 80$ phage tail have now been identified (15;

unpublished data). Since the gene coding for the major structural component of the phage tail has yet to be identified by an amber mutation, $\phi 80$ may employ more than 11 gene products in its tail assembly pathway.

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