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Genetic Studies of Coliphage P1

I. Mapping by Use of Prophage Deletions

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One hundred and ten amber mutants of coliphage P1 were isolated and localized into groups with respect to the existing genetic map by use of nonpermissive *Escherichia coli* K-12 strains lysogenic for P1 with deletions. These lysogens contain one of three types of deletion prophages: P1*cry* and its derivatives, P1*dlac*s, and P1*dpro*s. Fourteen such lysogens were tested for their ability to rescue the amber mutants which were then assigned to one of nine deletion segments of the P1 genome defined by the termini of the various prophage deletions. The relationship of the nine deletion segments with the published P1 map is described, two new segments having been added. The deletions of the 14 prophages overlapped sufficiently to indicate that the P1 genetic prophage map should be represented in circular form, which is consistent with the fact that P1 is normally a circular plasmid in the prophage state. The distribution of mutants into deletion segments is nonrandom for at least one segment. In addition, the deletion termini of the 14 defective prophages coincided in five out of nine regions separating the nine deletion segments. Various possible explanations are discussed for the nonrandom recurrence of these deletion termini, including the evidence for hot spots of recombination.

P1 is unique among coliphages in several respects. In addition to the prophage being an autonomous circular plasmid (10), P1 normally produces, during lytic infection, mature virions with at least three different isometric head sizes containing differing amounts of DNA (26; D. H. Walker, Jr., Ph.D. thesis, Univ. of Pennsylvania, Philadephia, 1966). As a prerequisite for studying the morphogenesis of P1, it is necessary to establish an extensive genetic map so that, in particular, the genes responsible for head size determination and DNA packaging can be identified and characterized.

The published genetic map of P1 (19, 23) includes *vir;* five *c* cistrons (21, 22) and 10 amber cistrons of Scott; and nine of 140 amber mutants isolated in this laboratory. This paper describes the deletion mapping of 110 of these 140 amber mutants.

The usefulness of lysogens containing deletion prophages for genetic mapping has been widely recognized and such defective lysogens have been used for λ (2), P22 (6), and Mu-1 (1). Such lysogens now exist for P1 also: there are strains carrying P1*cry* and its derivatives (23), P1*dlac*s (14; M. E. Rae, Ph.D. dissertation, Univ. of Chicago, 1971), and P1*dpro*s (Rosner, personal communication). In the work reported here (D. H. Walker, Jr., and J. Tweedy-Walker, Abstr. Annu. Meet. Am. Soc. Microbiol., Chicago, Ill., 12-17 May 1974, p. 236, V213), 14 nonpermissive *Escherichia coli* K-12 strains lysogenic for P1 deletion prophages of these three types were used to locate the 110 mutations with respect to the existing P1 map (19, 23). These 14 defective lysogens also permitted assignment of the 110 mutations to nine map segments determined by the prophage deletions.

MATERIALS AND METHODS

Phage strains. P1*kc* of Lennox (13), referred to subsequently as P1+, and P1*vir* (the supervirulent mutant P1*kc vir*^{s} which grows on strains lysogenic for P1) were gifts from S. E. Luria. Amber mutants of P1 were isolated as described below except for mutants from cistrons 1 through 10 which were generously supplied by June R. Scott. λ·C600 was a gift from M. Feiss.

Bacterial strains. The *E. coli* K-12 strains are described in Table 1. Since most of the defective prophages in P1*dlac* lysogens tended to segregate out in progeny cells (with the exception of PEO414), these strains were grown on media containing lactose instead of glucose and were tested for stability of the *lac* determinant as described below. The Su^{-} streptomycin-resistant strain of *Shigella dysenteriae*, Sh16

TABLE 1. *Escherichia coli K-12 strains*

Strain	Relevant description	Source and reference
DW101	Su$^-$(=*sup*$^+$)	This lab[a]
DW103	Su$^+$(*sup*D)	This lab[b]
C600	Su$^+$(*sup*E)	G. Bertani (3)
K140	Su$^-$	J. R. Scott (9)
K140(P1*cry*)	Su$^-$, *cam*^{r}	J. R. Scott (20)
RW347	Su$^-$	J. R. Scott (24)
ΔCM2	*cam*^{s} deletion of RW347(P1*cry*)	J. R. Scott (23)
ΔCM4	*cam*^{s} deletion of RW347(P1*cry*)	J. R. Scott (23)
ΔCM5	*cam*^{s} deletion of RW347 (P1*cry*)	J. R. Scott (23)
X111	Su$^-$, *pro-lac* Δ111	Rosner; Stodolsky (7)
PL0148	X111(P1*dlac*)	M. Stodolsky (15)
PL0200	X111(P1*dlac*)	M. Stodolsky (15)
PE0414	X111(P1*dlac*)	M. Stodolsky (M. E. Rae, Ph.D. dissertation, Univ. of Chicago, 1971)
1a	X111(P1*dpro*)	J. L. Rosner (18)
3a	X111(P1*dpro*)	J. L. Rosner (18)
4a	X111(P1*dpro*)	J. L. Rosner
6b	X111(P1*dpro*)	J. L. Rosner
8a	X111(P1*dpro*)	J. L. Rosner
11a	X111(P1*dpro*)	J. L. Rosner
14a	X111(P1*dpro*)	J. L. Rosner

[a] DW101 is a λ^s derivative of CA154 (5; a gift of R. Epstein) which gives a consistently high efficiency of plating for P1; CA154 was cured of λ by heteroimmune superinfection with λi^{434}.

[b] DW103 was constructed by isolating a *his*$^-$ mutant of DW101 and co-transducing *sup*D with *his*$^+$ using P1·CR63.

(14; a gift from S. E. Luria), was used with P1*am*$^+$ for assays and to distinguish clear and turbid plaques.

Media. A modification of L broth (WLB; 1% Trypticase [BBL], 0.5% yeast extract [Difco], 0.5% NaCl, 0.1% glucose, and pH adjusted to 7.4 with 1 N NaOH) was routinely used for growing bacteria. For growing P1*dlac* lysogens, WLB lac (WLB containing 0.1% lactose instead of glucose) was used. $CaCl_2$ and $MgSO_4$ (final concentrations 5 mM) were added to these media when used for adsorption mixtures, Ca^{2+} being required for P1 adsorption (4) and Mg^{2+} for increased P1 stability (Walker, unpublished data).

For routine P1 assays and for the preparation of stocks from confluent lysis plates, bottom agar consisted of WLB with 1.0% Difco or 0.8% Fisher agar ($CaCl_2$ and $MgSO_4$ were each added to a concentration of 5 mM just before pouring). Soft top agar consisted of 1% Trypticase (BBL), 0.5% NaCl, and 0.7% agar (Difco).

For testing the stability of the defective prophages in P1*dlac* lysogens, cultures of these strains used in experiments were streaked on Levine eosin methylene blue agar (BBL). In the experiments described, such cultures produced 90 to 100% *lac*$^+$ colonies.

P1 buffer (1 liter of distilled water containing 1.5 ml of 10 N HCl, 2.42 g of Tris, 10 g of CH_3COONH_4, 5 g of $MgSO_4 \cdot 7H_2O$; pH adjusted to 7.3 to 7.4 after autoclaving and cooling) was used for dilutions of P1.

Phage assays. DW103 was the Su$^+$ host used for assaying P1 amber mutants. DW103 or Sh16 was used for assays of P1*vir* and P1+. Prior to plating, the phage to be assayed were adsorbed on the host for 10 min at 37 C in the presence of Ca^{2+} and Mg^{2+} (three drops of log phase *E. coli* or six drops of Sh16 were used as the host; Sh16 was grown at room temperature for 3 days as suggested by June R. Scott, personal communication). Adsorption tubes were then transferred to 45 C, 2.5 ml of soft top agar was added to each, and the mixtures were poured onto the surfaces of plates containing 45 to 50 ml of bottom agar. Plates were incubated right side up at 37 C, and plaques could easily be scored on the following day.

Preparation of phage stocks. Stocks of P1+, P1*vir*, and P1 amber mutants were prepared using a modification of the confluent lysis plate technique (25). Single plaques were stabbed by toothpick and transferred to 1 ml of P1 buffer in a serology tube. One milliliter of an overnight culture of host cells concentrated twofold and resuspended in WLB containing 5 mM $CaCl_2$ and $MgSO_4$ was added to the serology tube and the mixture was placed at 37 C for 10 min for adsorption of phage on cells to occur. This phage-cell mixture was transferred to 45 C, 3 ml of soft top agar (containing 0.8% instead of 0.7% Difco agar) was added, and the tube contents (5 ml) were plated on fresh, nondried, prewarmed bottom agar plates. After about 7 h of incubation at 37 C, when plaques were contiguous, the semisolid top layer was harvested, using a glass rod, into a 10-ml glass beaker. One drop of DNase (1 mg/ml) and three drops of chloroform were added and the preparation was emulsified by vigorous pipetting. After sitting overnight at 4 C the mixture was centrifuged (at 35,000 rpm for 3 min at 4 C in an L3-40 ultracentrifuge, using an SW50.1

swinging bucket head) to pellet agar, unlysed cells, and cell debris. Titers of supernatant fluids thus obtained are between 10^{10} and 10^{11} PFU/ml. Most of the amber stocks used in the experiments described had revertant levels of $\leq 10^{-6}$.

Isolation of amber mutants. A high-titer ($\sim 10^{11}$ PFU/ml) stock of P1*vir* was treated with hydroxylamine, as described by Scott (19), and plated on DW103. P1*vir* was used because the resulting P1*vir am* mutants could overcome superinfection immunity when plated on defective lysogens that contain functional repressor. (Five P1*am* mutants were isolated after plating a mutagenized high-titer stock of P1+ on C600. These five mutants were used in marker rescue tests with defective lysogens which do not exhibit superinfection immunity.)

Tests for restricting and modifying ability of lysogens. Defective lysogens were tested for ability to restrict the growth of λ, using a method similar to that described by Rosner (16). Drops containing about 1.5×10^2 and 1.5×10^6 PFUs of λ·C600 were placed on overlays of the defective P1 lysogens and their isogenic controls. A decrease in titer of λ of about 10^{-3} on a lysogen as compared to its control was taken to indicate possession, by the lysogen, of restricting ability. The possession of modifying ability by each defective lysogen was shown when P1-modified λ grown on the lysogen being tested plated equally well on DW103 and DW103(P1CM).

Spot test for marker rescue. Using this method, defective lysogens were tested for ability to supply am^+ alleles to the 110 amber mutants. Soft agar overlays were made containing $\sim 2 \times 10^8$ cells of the desired Su^- defective lysogens, their isogenic controls, and our standard Su^+ host, DW103. A multiprong replicator was used to stamp three dilutions (10^6, 10^7, 10^8 PFU/ml) of each *vir am* mutant, from nylon replica chambers (Elesa, Milan, Italy) with 25 wells, onto each overlay. (Each stainless-steel replicator prong deposits a volume of about 0.005 ml.) P1+ and P1*vir* (10^8 PFU/ml dilutions) were also stamped on each overlay: P1+ to detect superinfection immunity and P1*vir* as a positive control. After overnight incubation at 37 C the plates were scored for PFUs. For each amber mutant tested, at least a 50-fold increase in PFUs obtained on a defective lysogen compared to those obtained on its isogenic control indicated the presence of the am^+ allele in that defective lysogen. Detection of such an increase was possible because (i) three concentrations of each amber mutant were stamped on any one defective lysogen and its isogenic control (as well as on the Su^+ host, DW103), and (ii) the small size of P1 plaques obtained under the plating conditions of this test allowed 50 to 100 plaques to be scored (or estimated) within a given spot.

Broth tests for marker rescue. This method was used both to determine the left terminus of the P1*dpro* lysogen 8a deletion and to determine placement of *vir*. In determining the deletion terminus, the *vir* amber mutants to be tested were placed in separate wells in duplicate replica chambers. One drop of 0.5 M $CaCl_2$ was added to each well. Lysogen 8a was added to ambers in one replica chamber and the isogenic control to ambers in the duplicate (for each the final cell concentration was $\sim 1 \times 10^8$/ml, the multiplicity of infection was ~15, and the total volume was 0.55 ml). Included as controls were P1*vir*, *vir am* 176 (known by spot tests to show rescue of the am^+ allele from lysogen 8a), and *vir am*108 (known not to show rescue of the am^+ allele from lysogen 8a). After 20 min at 37 C to allow adsorption, contents of each replica chamber were diluted 25-fold to a second replica chamber. After incubation of these second replica chambers for 60 min the cells were killed with chloroform and the phage were assayed on the Su^- host, X111.

A modification of this method was used in determining the placement of *vir*, by rescue of the vir^+ allele from defective lysogens. P1*vir* (multiplicity of infection of 5 to 10) was incubated 20 min with each of the defective lysogens ($\sim 5 \times 10^8$ cells/ml) in a replica chamber. The contents of the chamber were diluted 100-fold by replication to a second chamber, using the multiprong replicator. After incubation of the second replica chamber for 2 h at 37 C the cells were killed with chloroform and the progeny phage were plated on Sh16 and scored for percent turbid plaques.

RESULTS

Isolation of amber mutants. One hundred and forty mutants were isolated after hydroxylamine mutagenesis. As these were isolated at different times, they were labeled A through M (excluding I), 20 through 68, and 101 through 180. Mutants F, G, H, K, and M are P1*am* mutants, the remaining ones are P1*vir am* mutants. Of the 140 mutants isolated, 110 were found to be satisfactory for this work because they had low revertant levels. Nine of the 110 amber mutations have already been located on the P1 map in this laboratory (unpublished data) and by J. R. Scott (20).

Marker rescue by spot tests. For most amber mutants results were unambiguous, i.e., there was an estimated increase of at least 50-fold in PFUs on a defective lysogen compared to the number of PFUs (revertants) occasionally present on the isogenic control. Such an increase was scored as rescue of the am^+ allele from the defective prophage. An exception was P1*dlac* lysogen PL0148 which at first gave ambiguous results for several markers tested, but this ambiguity was found to be due to a segregation problem which was overcome by growing the lysogen in WLB lac prior to the tests. Further exceptions, giving repeatedly ambiguous results, were spot rescue tests of *vir am* mutants 4.7, 62, 117, 150, and 174 with P1*dpro* lysogen 8a. Results with these mutants are discussed below.

To summarize our results, use of the 14 defective lysogens in these marker rescue tests

with 22 P1 mutants already mapped by Scott (19, 23) divided the published P1 map (Fig. 1) into seven deletion segments (A, B, C, D, G, H, J) as defined by the termini of the prophage deletions. When the 110 mutants isolated in this laboratory were tested with the 14 defective lysogens, most of the amber mutations could be assigned to these seven deletion segments. The remaining mutations added two more segments (E and F), which did not have any mutants represented on the published map. Distribution of the 110 mutations into the nine deletion segments can be seen by comparing Fig. 1 and Table 2. When the prophage deletions in Fig. 1 are represented in circular form (Fig. 2), it is more easily seen that there is only one discrete deletion for each deletion prophage. The deletions are overlapping, indicating circularity of the P1 genome. The unlettered segment (*cam*^{r}) is a small addition of ~3% to the P1 genome (Ohtsubo, personal communication); it can be lost spontaneously during lytic growth of P1CM (11, 18) and is therefore not a stable component of the P1 genome. For this reason it is not included in our deletion segment designations.

Our results with the previously mapped mu-

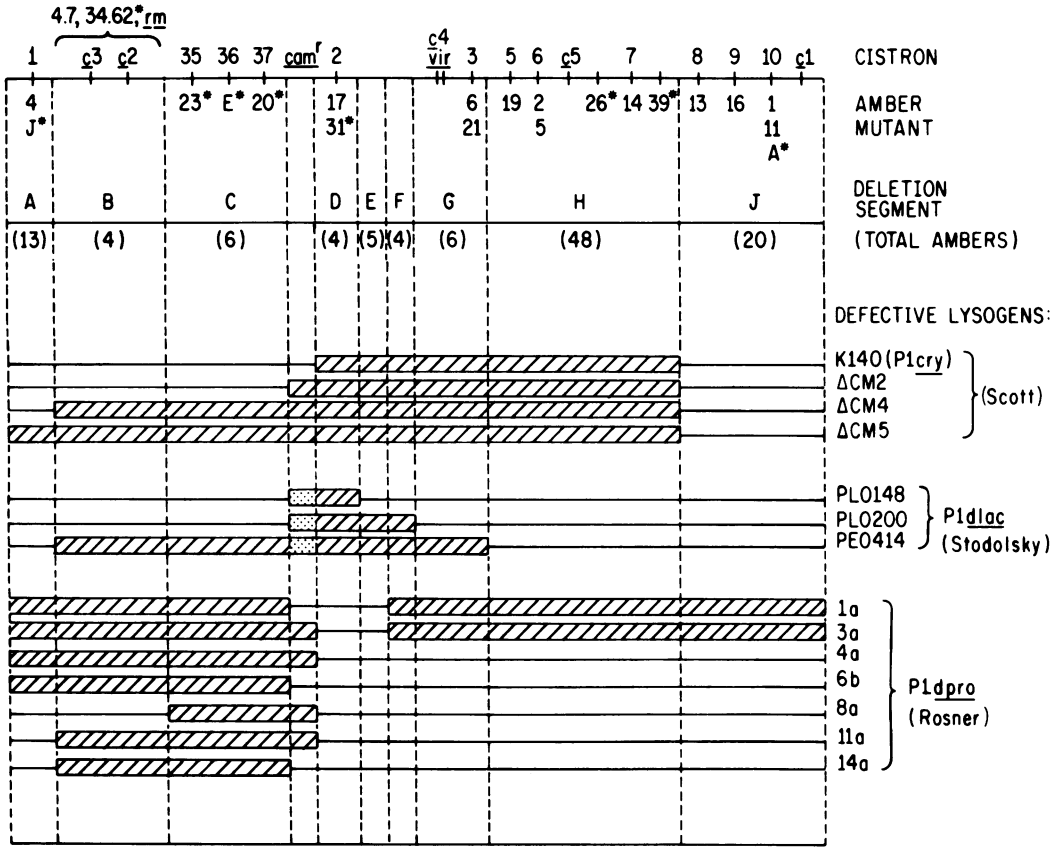

FIG. 1. *P1 genetic map showing point mutations and prophage deletions for 14 defective lysogens. The top line represents the current P1 genetic map published by Scott (19, 23); it is not drawn to scale and the order of the genes above the brace is not known. Amber mutants isolated by us and mapped by Scott in collaboration with us are indicated by an asterisk (*). Vertical dashed lines represent deletion termini (with respect to the P1 genetic map) and the division of the map into deletion segments (A through J, excluding I). The numbers in parentheses indicate those amber mutants assigned to each deletion segment; amber mutant designations are in Table 2. Each prophage deletion is labeled with the strain designation of the defective lysogen in which it is carried (see Table 1). For each defective lysogen, the cross-hatched bar indicates the prophage deletion, i.e., where there is no rescue; the solid line indicates those segments of the prophage from which mutants do show rescue. The cam*^{r} *region is an addition to the P1 genome and refers to P1cry and derivative deletion prophages and to the P1dpro deletion prophages; the stippled area for the P1dlac deletion prophages denotes that the cam*^{r} *region is not relevant to these strains. Details concerning the state of the deletion prophages (i.e., integrated or autonomous), superinfection immunity, restriction, and modification are given in Table 3.*

tants (Fig. 1) were in agreement with those obtained by Scott (23) with defective lysogens K140(P1*cry*), ΔCM2, ΔCM4, and ΔCM5; and our results with amber mutants 1.4, 2.17, 3.21, 10.1, and 62 were in agreement with those obtained by Rosner (personal communication) who had tested the P1*dpro* lysogens for rescue of the am^+ alleles of these mutants as well as for chloramphenicol resistance (cam^r), superinfection immunity, restriction, and modification (Table 3).

Detailed examination of results of marker rescue spot tests revealed that, on the basis of these tests alone, some of the deletion termini could not be established and some of the mutations could not be located in unique deletion segments. Thus the deletion terminus of defective lysogen 8a between deletion segments

TABLE 2. *Distribution of amber mutants among the nine deletion segments of Fig. 1*

Deletion segments	A	B	C	D	E	F	G	H	J	
Amber mutant designation	H 128 J 129 34 132 37 141 47 165 57 173 176	62 117 150 174	E 20 23 108 134 144	31 56 103 170	D 33 66 114 149	F G 38 49	35 118 127 143 155 171	B 32 101 125 151 K 39 102 126 154 M 40 107 130 156 21 42 109 133 157 22 45 110 135 160 26 51 113 138 162 27 53 115 139 168 28 67 119 140 179 29 68 120 142 180 30 121 148	A 106 36 111 43 112 46 131 48 136 54 137 58 147 64 152 104 175 105 178	
Total ambers (this lab)	13	4	6	4	5	4	6	48	20	110 (total)
Total ambers including Scott's	14	5	6	5	5	4	8	52	24	123 (total)

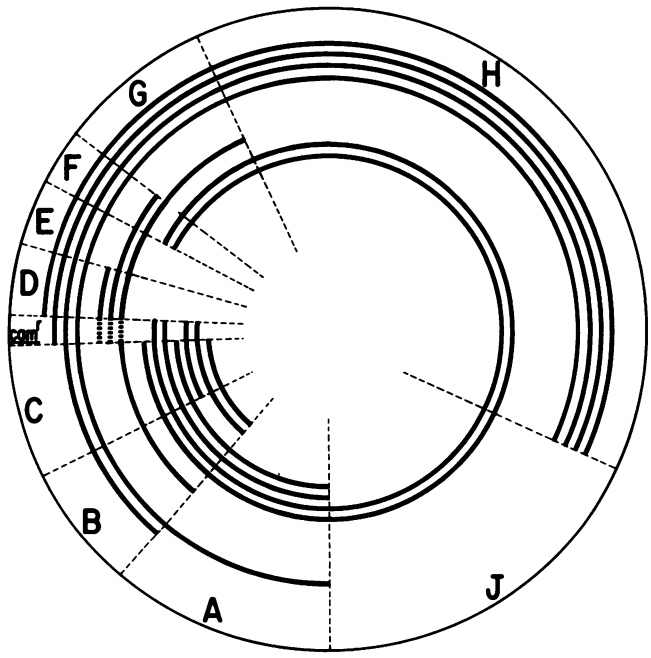

FIG. 2. *The genetic map shown in Fig. 1 redrawn (without point mutations) to emphasize the overlapping deletions which indicate circularity of the P1 genome. The thick lines represent deletions and correspond to the cross-hatched bars in Fig. 1.*

B and C was determined by marker rescue broth tests (next section) and the left deletion termini of ΔCM4, PE0414, 11a, and 14a between deletion segments A and B were determined in the following way. These four defective lysogens do not exhibit either P1 restriction or modification (Table 3) and are therefore phenotypically r^-m^-. The genes responsible for the restriction and modification phenotype must then be located in segment B since lysogen 8a is phenotypically r^+m^+. Genes *c*2 and *c*3 are located in segment B or C because Scott (23) found rescue of $c2^+$ and $c3^+$ from ΔCM2, but not from ΔCM4. Since Rosner has shown that *c*2 and *c*3 are required for modification (17), it is unlikely that these genes are present in segment A and, since 8a is r^+m^+, it is unlikely that *c*2 and *c*3 are in segment C. In addition, since $c2^+$ and $c3^+$ are not rescued from ΔCM4 (which is phenotypically r^-m^-), it is unlikely that the *c*2 and *c*3 genes are present in PE0414, 11a, and 14a either. For these reasons we have located the left deletion terminus for ΔCM4, PE0414, 11a, and 14a to the left of genes *c*2 and *c*3 and, when the deletion terminus of lysogen 8a was established between deletion segments B and C as described below, genes *c*2 and *c*3 were placed in segment B.

Of the five P1*am* mutations, *am*H was located in deletion segment A because the am^+ allele is rescued from ΔCM4 but not from ΔCM5, both of which are defective lysogens which do not exhibit superinfection immunity (Table 3). However, the remaining four P1*am* mutations (*am*F, *am*G, *am*K, and *am*M) could not be located in a unique deletion segment because the P1*lac* and P1*dpro* defective lysogens (except 1a and 3a) exhibit superinfection immunity as shown by the inability of P1+ and the ability of P1*vir* to grow on these defective lysogens. These mutants were located within the region including deletion segments F through H by use of defective lysogens K140(P1*cry*), 1a, and 3a which do not exhibit superinfection immunity (20; Rosner, personal communication). The four mutants were subsequently placed in deletion segments F and H (Table 2) by genetic crosses (manuscript in preparation).

Marker rescue by broth tests. These tests were performed to ascertain the left terminus of defective lysogen 8a. This would then define the demarcation between deletion segments B and C.

In spot tests, defective lysogen 8a showed am^+ allele rescue for mutants in deletion segment A (Table 2), but not for mutants in segment C. Spot test results for 8a with *vir am* mutants 4.7, 62, 117, 150, and 174 were ambiguous, i.e., no more than a 10-fold increase in PFUs above background was observed and results varied in repeated tests. In spot tests the am^+ alleles of these five mutants are unambiguously rescued from defective lysogen ΔCM2 but not rescued from defective lysogen ΔCM4. Therefore these five mutations are located in the vicinity of the left terminus of 8a. Unambiguous results for these five mutants concern-

TABLE 3. *Properties of defective lysogens*

Strain	Superinfection immunity[a]	Restriction[b]	Modification[c]	Integrated plasmid[d]
K140(P1*cry*)	−	+	NT[e]	+ (19, 20)
ΔCM2	−	+	+	+ (20)
ΔCM4	−	−	−	+ (20)
ΔCM5	−	−	NT	+ (20)
PL0148	+	+	NT	(−) (Rae, Ph.D. thesis)
PL0200	+	+	NT	(−) (Rae, Ph.D. thesis)
PE0414	+	−	−	(+) (Rae, Ph.D. thesis)
1a	−	−	−	+ (Rosner, personal communication)
3a	−	−	−	+ (Rosner, personal communication)
4a	+	−	−	(−) (Rosner, personal communication)
6b	+	−	−	(−) (Rosner, personal communication)
8a	+	+	+	(−) (Rosner, personal communication)
11a	+	−	−	(−) (Rosner, personal communication)
14a	+	−	−	(−) (Rosner, personal communication)

[a] These results agreed with those of Scott, Stodolsky, and Rosner for their strains (Table 1).
[b] These results agreed with those of Scott and Rosner for their strains.
[c] These results agreed with those of Rosner for his strains.
[d] Parentheses around symbols, i.e. (+) or (−), signify results based on suggestive evidence only. Figures in parenthesis denote references.
[e] NT, Not tested.

ing rescue of their *am*$^+$ alleles from 8a were obtained in broth tests.

The results are shown in Table 4. The second and third columns indicate the burst size of *am*$^+$ progeny of each mutant after growth on 8a and on the isogenic control, X111; the *am*$^+$ PFUs were scored on X111 and the burst size was calculated from the number of infected cells. Since only revertants can grow on X111, the ratio of bursts (column 4) indicates whether or not rescue has occurred (column 5). The negative control, *vir am*108 (which showed no rescue in spot tests), gave a ratio of 0.13. Theoretically, the ratio should have been ~1, but P1*vir* itself does not give as high a burst on 8a as it does on X111, so all *vir am* mutants are probably affected similarly. The positive control, *vir am*176 (which showed rescue unambiguously in spot tests), gave a ratio of 27 which is more than 200 times higher than that of the negative control. Of the five mutants tested, the one giving the lowest ratio (*vir am*117) has a ratio which is 100 times that of the negative control. It was therefore concluded that, for all five mutants, rescue of the *am*$^+$ alleles from 8a does occur and that the terminus of 8a demarcates deletion segments B and C (Table 2 and Fig. 1).

The comparative burst sizes on 8a alone are about two orders of magnitude greater for the five mutants than for the negative control. However, the burst sizes are two orders of magnitude less than for the positive control. The reason for these results is unclear but they do correlate with the spot tests giving ambiguous results. The variabilities in the ratios reflect the fluctuating revertant levels (column 3).

Marker rescue broth tests were also used to determine location of *vir*, which had previously been placed between amber mutants 2.17 and 3.6 (19). In these broth tests, placement of *vir* in relation to the deletion segments (D, E, F, and G) in this region was determined by rescue of the *vir*$^+$ allele from the 14 defective lysogens. Production of turbid plaques after incubation of P1*vir* with each defective lysogen indicated rescue of the *vir*$^+$ allele. In this experiment (Table 5) *vir*$^+$ was rescued from defective lysogens PL0148 and PL0200 but not from PE0414, 1a, or 3a, thus placing *vir* in deletion segment G and, from Scott's data (19), to the left of amber 3.6. Results with the remaining defective lysogens were as expected: namely, *vir*$^+$ was rescued from defective lysogens 4a, 6b, 8a, 11a, and 14a (which are known from spot tests not to be deleted for deletion segments D, E, F, and G) and *vir*$^+$ was not rescued from defective lysogens K140(P1*cry*), ΔCM2, ΔCM4, and ΔCM5.

DISCUSSION

One hundred and twenty-three amber mutants (including those of Scott) have now been mapped into nine deletion segments. If *vir* and the five *c* mutants (21, 22) are included, the total number of mutants for P1 is 129. The calculations which follow are based on this number. The DNA of normal infectious P1 virions has a molecular weight of about 60×10^6 and, if one excludes the large terminal redundancy (10), the P1 genome would have a molecular weight of about 53×10^6. This amount of DNA could reasonably code for at least 70, and

TABLE 4. *Rescue of am*$^+$ *allele of mutants from defective lysogen P1dpro 8a*

Vir amber mutant	Burst size[a] on 8a	Burst size[a] on X111	Ratio of bursts (8a/X111)	Rescue of *am*$^+$
4.7	1.9×10^{-2}	8.5×10^{-5}	224	+
62	1.2×10^{-2}	9.1×10^{-6}	1,319	+
117	3.5×10^{-2}	2.5×10^{-3}	14	+
150	4.5×10^{-2}	8.7×10^{-5}	517	+
174	1.9×10^{-2}	4.8×10^{-6}	3,958	+
176[b]	2.5×10^{0}	9.3×10^{-2}	27	+
108[c]	1.6×10^{-4}	1.2×10^{-3}	0.13	–
P1*vir*[d]	20	92	0.22	–

[a] Burst size of *am*$^+$ progeny grown on strain indicated and calculated from number of infected cells; PFUs obtained from assay on X111.

[b] Positive control from marker rescue spot tests.

[c] Negative control from marker rescue spot tests.

[d] Three independent experiments confirmed that P1*vir* has a lower burst on 8a than on X111; P1*vir am* mutants are affected similarly as indicated by the results of P1*vir am*108.

TABLE 5. *Rescue of vir⁺ from defective lysogens*

Strain	% Turbid plaques[a]	Rescue of *vir⁺*
K140(P1*cry*)	<0.04	–
ΔCM2	<0.07	–
ΔCM4	<0.07	–
ΔCM5	<0.05	–
PL0148	0.61	+
PL0200	1.72	+
PE0414	<0.04	–
1a	<0.04	–
3a	<0.04	–
4a	2.43	+
6b	3.13	+
8a	6.57	+
11a	2.90	+
14a	1.82	+

[a] After infection with P1*vir*; where there were no turbid plaques, i.e., no rescue, at least 1,500 plaques were scored.

no more than 90, genes. Assuming that mutations have occurred randomly and are equally detectable and that all genes are essential, we should theoretically have found 59 to 68 genes. If we add *vir* and the five *c* genes to the number of cistrons we found by complementation tests for all the amber mutants (manuscript in preparation), the actual number of cistrons found is 50 to 55. This would account for no less than 74% and no more than 93% of the total P1 genome. In other words, only 7 to 26% of the genome is theoretically unaccounted for by mutations. These calculations are within reasonable expectations because both mutation hot spots and nonessential genes probably exist. It should be noted in this respect that genes not essential for lytic infection would not have been detected by the isolation procedure used for obtaining amber mutants.

Fourteen defective lysogens were used for the deletion mapping which divided the genetic prophage map of P1 into nine deletion segments. Several points of interest emerge from this mapping data. First, the prophage deletions overlap (Fig. 2), indicating that the P1 genetic prophage map should be represented in circular form. This is consistent with the fact that the P1 prophage exists as a circular plasmid (10).

Second, distribution of mutants within deletion segments (Table 2) does not appear to be random. Segment H has 52 of the 123 mutants (42%), which represents about 40% of the total known cistron content of P1 (manuscript in preparation). The reason for there not being any deletion termini which would subdivide segment H into smaller segments is not clear. It may be related to the fact that we have tested only three types of defective lysogens (i.e., those carrying P1*cry* and derivative prophages, P1*dlac* prophages, and P1*dpro* prophages) and that there may be selective pressure for these particular lysogens in terms of integrated versus autonomous prophages. For example, it can be seen by examining Fig. 1 (and Table 3) that defective lysogens having autonomous deletion prophages lacking deletion segment H (or G or J) do not occur. A larger sampling of defective lysogens and extensive data on gene functions (e.g., concerning maintenance of lysogeny) would be necessary to test the validity of the above speculations.

Third, the 14 defective lysogens defined only nine deletion segments for the P1 genome, due to recurrence of deletion termini; i.e., there are five regions on the genetic prophage map where two or more deletion termini occur (Fig. 1). Four defective lysogens have a terminus between deletion segments A and B (this region being designated A-B); 11 have a terminus at C-D; two have a terminus at E-F; four have a terminus at H-J; and three have a terminus at J-A. The apparently recurring terminus of four deletion prophages at H-J is deceptive; i.e., since the ΔCM lysogens were derived from the integrated P1*cry* prophage by selecting for loss of *cam*^{r}, no change in the position of this terminus was likely to be found in its derivative deletions (23). The recurrence of termini at the other four regions is surprising since we know that we have found 50 to 55 of the P1 genes, corresponding to 74 to 93% of the P1 genome. Several explanations for these recurrences are possible. (i) The reason for the recurrence of termini of 1a and 3a at E-F is unclear and may simply be due to an unusual coincidence since it occurs between two closely linked gene loci (manuscript in preparation). (ii) The recurrence of termini at the remaining three regions (A-B, C-D, and J-A) is striking because, in these regions, termini for two or more of the three deletion prophage types occur, e.g., the termini for ΔCM4, PE0414, 11a, and 14a coincide at A-B. These recurrences could conceivably result from scattering of the termini within regions but with the theoretical limitation that only 7 to 26% of the P1 genome is unaccounted for by mutations. (iii) The recurrence of termini at A-B, C-D, and J-A could also be explained by high negative interference suggesting that these regions (or sites) could be recombination hot spots. Again, this could apply to any or all of

these three regions. Hertman and Scott (8) found that, in the absence of vegetative recombination promoted by the host *rec*A-*rec*B pathway, there is an additional recombination system affecting P1 which is more active between *vir* and amber mutant 1.4 than to the right of *vir* (see linear map, Fig. 1). The A-B and/or C-D regions could be the locus/loci of its action.

The large number of recurring termini at C-D is particularly striking and there is some evidence that this region may be a recombination hot spot rather than a region of nonessential genes. The evidence is that C-D is known to be a region where recombination can occur because it is where *cam*, which is an addition to the P1 genome (11), is located and *cam* can easily be lost from this position. In addition, the coincidence of termini at C-D is not due to insertion of *cam* per se because the two autonomous P1*dlac* prophages, which have never been involved with *cam* insertion, each have a deletion terminus at C-D.

Further support for the recombination hot spot hypothesis applying to a region where termini coincide comes from the following observation. Lee et al. (12) have recently found that P1 plasmid DNA has an inverted repeat which is about 620 base pairs in length. From heteroduplex mapping they have found that the DNA of both plasmids and virions is heterogeneous in that a segment of about 3,000 base pairs (equivalent to 2 to 3% of the P1 genome) is itself inverted in about half of the molecules, presumably due to internal reciprocal recombination between the inverted repeat sequences on either side of the segment. Such a situation could lead to extensive localized recombination in vegetative genetic crosses. Ohtsubo (personal communication) has found, from heteroduplexed P1 plasmid DNAs, that this region with the inverted repeat is about 25 to 30% of the distance around the plasmid circle from the *cam* addition loop. This region could occur on either side of the *cam* region, so it is possible that the inverted repeat occurs between A and J since about 20% of the mutants were found in segments A, B, and C. If the inverted repeat occurs at A-J, it would explain why the ends of the published vegetative map in linear form are represented at this location. Solid evidence for these speculations awaits correlation of P1 genetic and physical maps.

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