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Genetic Studies of Coliphage P1
III. Extended Genetic Map

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An extensive genetic map of coliphage P1 has been constructed for 113 amber mutants, using primarily a modification of the conventional complementation spot test. These spot tests failed to classify the mutants into cistrons, but when they were quantitated they permitted assignment of the mutants into 10 linkage clusters. Furthermore, a linear order could be deduced for most of the mutants within each cluster. This strongly suggested that recombination was the predominant event generating plaques and that, for the practical purpose of rapid genetic mapping, such spot tests could be considered as a series of two-factor crosses. Six of the 10 linkage clusters correlated with the P1 genetic map established by Scott (1968). The locations of the remaining four clusters were determined by three-factor crosses and by prophage deletion mapping. The nonrandom occurrence of termini for 14 deletion prophages, which we established previously (Walker and Walker, 1975), and the coincidence of these termini with five out of the ten regions demarcating the linkage clusters are discussed. Complementation tests in liquid frequently gave ambiguous results. Therefore, cistron designations were not assigned.

Since the discovery of conditional lethal mutations, extensive genetic maps have been constructed for a considerable number of bacteriophages. Among temperate phages alone, λ (10), P22 (2, 18), Mu (13), and P2 (1) are the subjects of continued genetic study because of their individually unique properties. Phage P1, although widely used as a general transducing phage for fine structure mapping of the *Escherichia coli* chromosome, has itself only been the subject of somewhat sporadic interest over the years primarily because of considerable technical difficulties in working with it. The elegant proof by Ikeda and Tomizawa (14) that the P1 prophage exists as an autonomous plasmid has generated considerable interest in the control of establishment and maintenance of the lysogenic state (24-26, 28) and, recently, in the control of P1 prophage DNA replication (5).

As regards the lytic cycle of P1, we wish to understand those events that control and determine head size formation and DNA packaging. Our interest derives from the fact that P1 lysates contain mature virions with at least three different isometric head sizes (33). There is convincing evidence that the two virions with the largest head sizes (only one of which is infectious) can contain less than full heads of DNA (33). Since P1 DNA is circularly permuted, this is at variance with the Streisinger head-full hypothesis (31) for T4, which has also been invoked to explain the encapsidation of DNA for P22 (32) and P1 (21). To analyze the morphogenetic pathway of P1 virion assembly, an extensive genetic map, from which the appropriate conditional lethal mutants can be studied, will clearly be useful.

Scott (24) generated the first genetic map of P1 and it provides the framework upon which we have positioned most of our mutations. The isolation and mapping of 110 amber mutants into nine deletion segments by prophage mapping has been described previously (34). This paper reports our finding that, for P1 amber mutants, a modification of conventional spot complementation tests showed primarily recombination. Using these tests, we were able to order the mutants into 10 linear arrays, which we call linkage clusters, and within each cluster an unambiguous linear order could be deduced for many of the mutants. Six of the linkage clusters could be positioned on Scott's first map by including her mutants in the spot tests. A seventh cluster was added by deletion mapping (34) and by Scott's three-factor crosses (27); the remaining three clusters were added by three-factor crosses described in this paper.

Liquid complementation tests gave a considerable number of ambiguous results so that the assignment of mutants to cistrons was not possible.

MATERIALS AND METHODS

Phage strains. The 113 P1 amber mutants used

for genetic mapping were previously described by Walker and Walker (34). Mutants with letter designations, e.g., *am*A, have been changed to numbers, as indicated in the legend to Fig. 1. P1 amber mutants 1.4, 2.17, 3.6, 3.21, 4.7, 5.19, 6.2, 6.5, 7.14, 8.13, 9.16, 10.1, and 10.11 were generously provided by June R. Scott. The numbers preceding the decimal points refer to Scott's assignment of cistron numbers 1 through 10 (24). P1*vir* is the supervirulent mutant P1*kc vir*^{s} of Sarkar, which grows on strains lysogenic for P1 (24). Most tests were done using *vir am* mutants, but the *vir* notation is used only in three-factor crosses where the distinction is important.

Bacterial strains. The Su$^+$(*supD*) and Su$^-$ hosts used were *E. coli* K-12 strains DW103 and DW101 (34). A polarity-suppressing (*suA*) *E. coli* K-12 strain, DM1013 (20), obtained from M. Sunshine, was used for some complementation tests in broth. The Su$^-$ streptomycin-resistant strain of *Shigella dysenteriae*, Sh16, was used to distinguish clear and turbid plaques of P1*am*$^+$ recombinants from three-factor crosses (24).

Media, phage assays, and preparation of phage stocks. Media, phage assays, and preparation of phage stocks were described previously (34).

Cross-streak test for "complementation." A modification of the cross-streak test of Scott (24) was used. The surface of a soft-agar overlay containing $\sim 2 \times 10^8$ cells of an Su$^-$ strain (DW101 or Sh16) was dried, and a loopful of an amber mutant ($\sim 10^8$ or 10^9 PFU/ml) was streaked down the plate. When this had dried, loopfuls of this and other amber mutants (at the same concentrations) were streaked across it. After overnight incubation at 37°C, mutants that complement should produce an area of clearing at the intersection of the streaks. However, contrary to expectation, these tests showed primarily recombination rather than complementation (see Results), as did the spot tests described next.

Spot test for "complementation." A total of 2×10^8 PFU of one amber mutant was plated in a 2.0-ml soft-agar overlay with 5×10^8 Su$^-$ DW101 cells, giving a multiplicity of infection (MOI) of ~0.4. The overlays were allowed to harden at room temperature for 40 to 60 min (the first 10 min with the lids off), and then phage droplets were deposited from grid wells using a multiprong replicator as previously described (34). Twenty-four different amber mutants plus one control (the same mutant as in the background) were stamped onto the overlay. The test was quantitative in that the number of PFU deposited in any given spot gave an approximate MOI of 0.4 with respect to the host cells within the area of the spot. After overnight incubation at 37°C, extensive cross comparison was made of the spots on the same and on different overlays: P1 plaques are quite small (but discrete) under these plating conditions and >500 PFU are required to produce a confluent lysis spot; when there are ≤500 PFU, twofold differences are easily distinguishable. With these tests, as with cross-streak tests, most PFU are probably formed as a result of recombination leading to *am*$^+$ phage. The validity of these tests depends on the quantitation possible. Accurate dilutions of recently titered stocks must be used. The droplets on the replicator prongs are visually inspected for uniformity of size before deposition onto the overlay. Although the droplet size can vary by 20%, this is an extreme variation based on how slowly or rapidly the replicator is lifted from the grid well. This was the most limiting step in quantitating the procedure and was minimized by having the same person perform the replicating for a given set of plates on a given day.

After mutants were assigned to linkage clusters defined by these tests (see Results), further testing of mutants within each cluster was done using the original and a 10-fold dilution of each mutant for replicating onto overlays, resulting in MOIs of ~0.4 and ~0.04. The amber in the Su$^-$ background remained at MOI ~0.4. Countable numbers of PFU were obtained in the spots where the higher and/or lower dilutions were applied.

Broth test for complementation. DW101 was grown to log phase ($\sim 2 \times 10^8$ cells/ml) in WLB (34) and chilled. $CaCl_2$ was added to a final concentration of $\sim 10^{-2}$ M, and the two mutants to be tested were added. The final cell concentration was $\sim 1.5 \times 10^8$/ml, and the MOI for each phage was 5 to 10. As controls, each mutant alone (MOI, 10 to 20) was used. After 15 min at 37°C (required for optimal phage adsorption), anti-P1 serum was added at a final k value of 2/min. After 5 min at 37°C, the contents of each tube were diluted 1,000-fold into 37°C broth. After 60 min (or 80 min in some tests) at 37°C, chloroform was added and the lysate was assayed on DW103 for total progeny and on Sh16 for *am*$^+$ recombinants. The total time was 80 to 100 min. Unadsorbed phage was assayed at $t = 15$ min before addition of the anti-P1 serum. Usually 80 to 95% of the phage were adsorbed. DM1013 (*suA*) was used as the host in some complementation tests in an attempt to determine if polarity existed.

Three-factor crosses. The method was the same as for the broth test for complementation, except that Su$^+$ strain DW103 was used as the host and the wild-type recombinants were selected by plating on Sh16 and scored as turbid or clear to determine the percent that carried *vir* as the unselected marker. The rationale of this procedure has been described by Scott (24).

RESULTS

"Complementation" spot tests. For many phages, e.g., λ (3), T4 (6), T5 (9), and T1 (7), amber mutants have been assigned to cistrons by putting overlapping droplets of any two mutants onto an overlay of Su$^-$ cells. In these tests the criterion used to decide that the mutants are not in the same cistron is that the region of overlap will be clear due to complementation. However, a clear region due to complementation in the overlap area (or in the spot area in our test) would only be obtained if the concentration of mutants used is sufficiently high, because at lower concentrations, although complementation may occur in the isolated cells which happen to be coinfected with the two

mutants, formation of a visible plaque would require surrounding cells to be coinfected with one of each type of mutant. Thus, at lower phage concentrations one would not expect to obtain visible plaques on the Su^- lawn unless a recombinational event had occurred leading to the formation of wild-type (am^+) phage, and such visible plaques would indicate recombinational events, even though some complementation may be occurring in the spot also. Such recombination would indicate that the mutants are in different cistrons, unless high frequencies of intracistronic recombination occur. (In similar spot tests with P22 [32], intragenic recombination frequencies as high as 10% have been obtained.) Absence of clearing or visible plaques in the spots at both phage spot concentrations (MOI, ~0.4 and ~0.04) would indicate that the mutants being tested are in the same cistron.

Initially, the phage backgrounds that we used were representatives of each of the 10 cistrons designated on Scott's genetic map (24). Mutants from the different cistrons did not always show complete clearing, notably *am*5.19 on *am*6.2 (and vice versa) and *am*9.16 on *am*10.1. (*am*5.19 and *am*6.2, and *am*9.16 and *am*10.1, are known to be closely linked from three-factor cross data [24].) Also, there was less than complete clearing between *am*7.14 and *am*5.19 or *am*6.2 and between *am*2.17 and *am*3.6.

This suggested that, with P1, perhaps we were detecting primarily recombination and not complementation. To test this hypothesis, three two-factor crosses were done in broth with DW103 and DW101 (isogenic Su^+ and Su^- strains): *am*3.6 × *am*9.16, *am*3.6 × *am*10.1, and *am*9.16 × *am*10.1. The Su^+ cells were permitted to lyse, the progeny were plated on Su^+ and Su^- overlays, and the am^+ recombinants were found to be 8.7, 9.6, and 0.23%, respectively. The infected Su^- cells were not allowed to lyse, but were diluted and plated on Su^+ and Su^- overlays as infective centers. Considerably fewer PFU appeared on the Su^- overlays: (PFU on Su^-) × 100/(PFU on Su^+) gave 9.3, 8.2, and 0.2%, respectively. Results from infection of Su^+ cells measured percentage of am^+ recombinants, and results from infection of Su^- cells measured percentage of bacteria producing am^+ recombinants. These two sets of observations need not be strictly comparable, but the fact that the percentages are so similar in this instance suggested that an Su^- cell coinfected with ambers from different cistrons cannot form a plaque unless a recombinational event leading to the production of am^+ phage has occurred. Therefore, if quantitated to the extent described in Materials and Methods, these spot tests could be considered as two-factor crosses. (From the spot tests, plugs of agar from both clear and less than clear spots were removed and the progeny phage had the same efficiency of plating on Su^+ and Su^- hosts, showing that these phages were am^+ recombinants; this result would be expected, however, even if some complementation were occurring, since am^+ phage would rapidly outgrow *am* mutants within spots on Su^- lawns.)

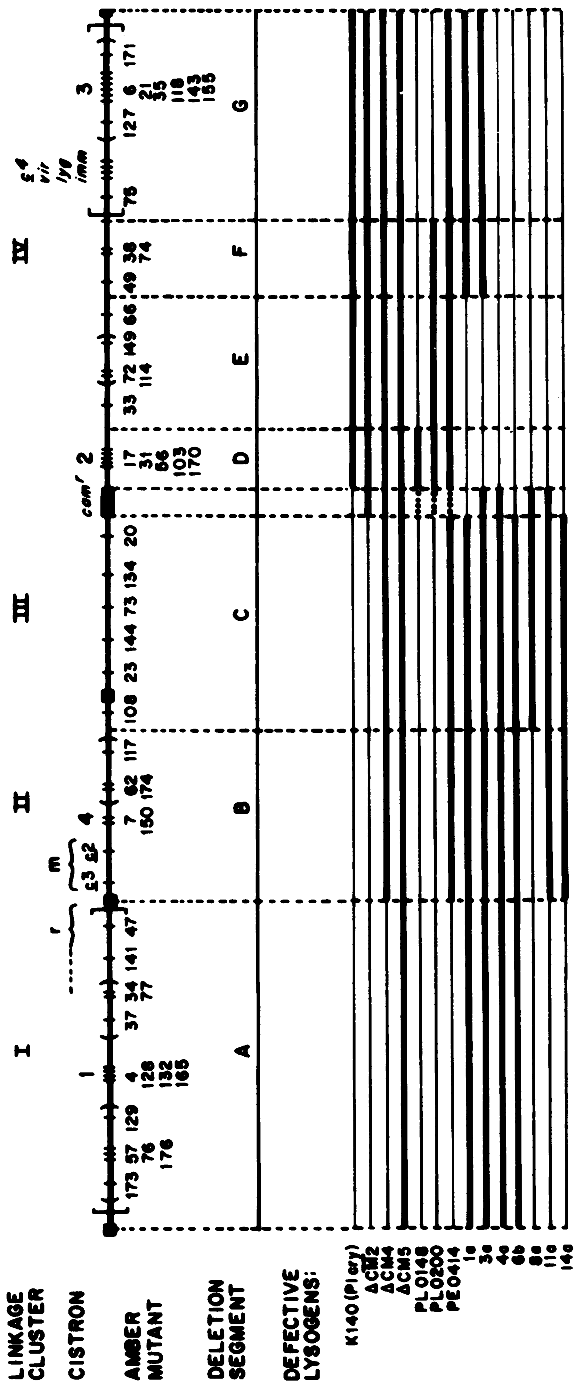
Results of spot tests obtained using our 100 P1 mutants and 13 representatives of Scott's 10 cistrons were unusual in that the mutants could be grouped into 10 linkage clusters (see Fig. 1, I through X, *am*8.13 being the sole member of "cluster" IX). We define a linkage cluster as being comprised of those amber mutants that, in the spot tests, show less than complete clearing with each other in all pairwise combinations and that in many cases can be linearly ordered as described below.

Mutants in different linkage clusters show complete clearing with each other in those spot tests. If lower phage concentrations are used, these mutants can show less than complete clearing, but the degree of clearing is about the same for mutants in one cluster with respect to mutants in any other cluster; hence, no linear ordering of mutants is possible by this method using mutants in different clusters.

About 75% of our mutations showed linkage with Scott's amber mutations, which had been linearly ordered by three-factor crosses. By our tests the 10 cistrons mapped by Scott were in different linkage clusters, except for 2 and 3; 5, 6, and 7; and 9 and 10. Scott could not determine the order of genes 5 and 6 with respect to the other genes on her map through the use of three-factor crosses. One advantage of our spot test was that this order could be established, because the data obtained in this test were derived from a considerably larger number of mutants than were used in her crosses.

The remaining 25% of our mutations constituted four different linkage clusters, none of which were linked to mutations on Scott's map. These were inserted into the map as described in the section on three-factor crosses.

Once a group of mutants had been assigned to a given linkage cluster, we determined their order within that linkage cluster as shown by the example in Table 1. In the case of cluster III, which has five mutants, two dilutions of each of the five were spotted on the five different phage-Su^- backgrounds. For each mutant, the relationship of the other four was established by comparing the relative numbers of PFU in each spot: presumably, the fewer the


LINKAGE CLUSTER
CISTRON
AMBER MUTANT
DELETION SEGMENT
DEFECTIVE LYSOGENS:
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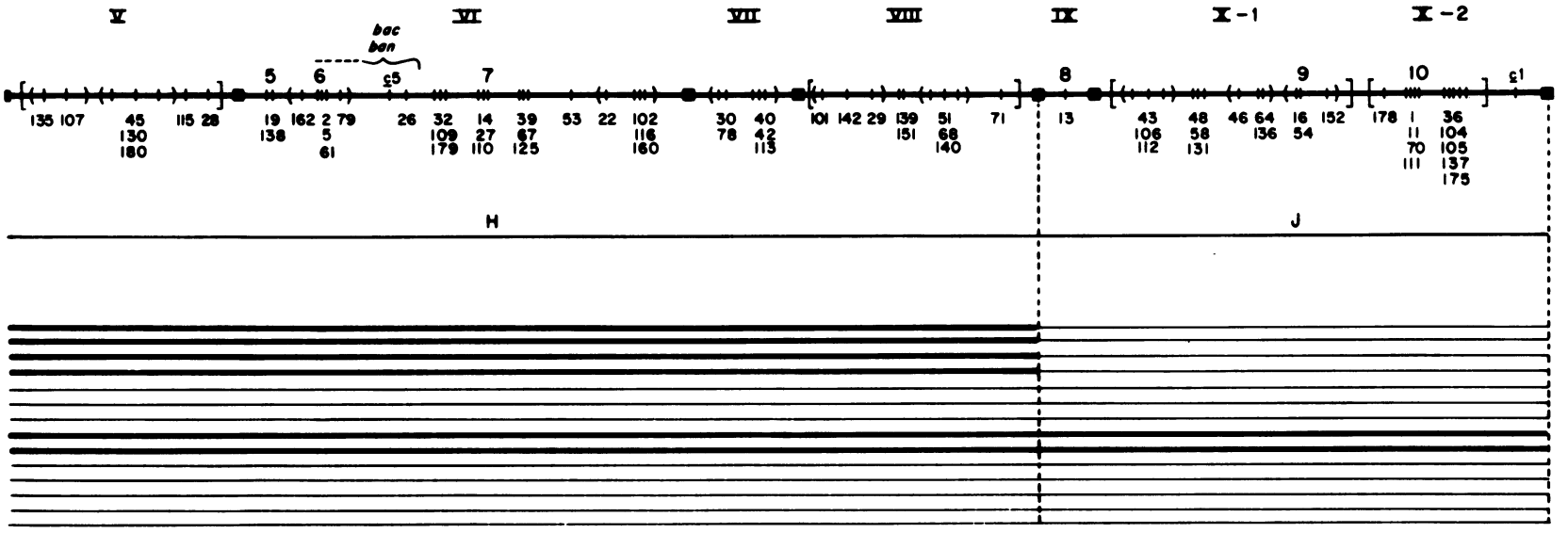

FIG. 1. *Genetic map of P1 based on two-factor spot tests and three-factor crosses. The spot tests divide the map into 10 linkage clusters (I to X, X being subdivided into two parts; see text). The regions demarcating the 10 linkage clusters are represented by solid blocks. The cistron designations above the map line include the original 1 to 10 of Scott (24). Previously published (4, 5, 27-29, 35) cistron designations, 34, 35, 36, 37, 56, and 66, which were used for amber mutants 62, 23, E (=73), 20, 32, and 43 respectively, are not justified (see text) and are omitted from this genetic map. Other designations are vir and the five c genes of Scott (24-26), of which c2 and c3 have been shown to be modification deficient (22) and are labeled m; r, the restriction gene which maps somewhere in deletion segment A (Cowan and Scott, personal communication; J. T. Walker, unpublished data); cam^r, which refers to the location where the CM marker in the P1CM strain of Kondo and Mitsuhashi (15) is inserted; lyg, which causes unstable lysogeny (28); the bac and ban mutations of D'Ari et al. (5), which map in the vicinity of c5 and to the left of am32 in linkage cluster VI; and imm, which is the location of immunity specificity (4). The orientation of groups of amber mutants within parentheses or within brackets with respect to mutants outside these groups is unknown. (Orientation of the group of mutants in deletion segment G is unclear and may be the reverse of that shown [West and Scott and Mural and Vapnek, personal communications].) Where hash marks, denoting relative positions of mutants, are very close together (and the mutant designations are placed below each other in the same column), little or no recombination occurred in spot tests and no complementation was found in liquid tests. Where the hash marks are not close, mutant designations in the same column indicate that the order of these mutants is not known (e.g., am51, am68, and am140 in linkage cluster VIII). The spacing of the hash marks is not meant to indicate either genetic or physical distance. Deletion segments A through J are defined by the termini of 14 deletion prophages described previously (34); the heavy part of the lines represent the deletions in the prophages for each of the defective lysogens. Note that the majority of deletion termini coincide with 5 out of the 10 demarcation areas separating linkage clusters. By agreement with Scott, 10 mutants previously given letter designations (11, 27-29, 34) have now been changed to numbers as follows: 70 = A, 71 = B, 72 = D, 73 = E, 74 = F, 75 = G, 76 = H, 77 = J, 78 = K, and 79 = M. In addition, am21 (in cistron 6) has been changed to am61 because am21 had already been used by Scott (24) in cistron 3.*

TABLE 1. *Ordering of amber mutants in linkage cluster III by two-factor plate crosses (spot tests)*[a]

Mutants
23 - 144 - (73, 134) - 20 - - - (31, 108)
144 - (23, 73) - 134 - 20 - - - (31, 108)
73 - (134, 144) - (20, 23) - - (31, 108)
134 - 73 - (20, 23, 144) - - - (31, 108)
20 - (73, 134) - (23, 144) - - (31, 108)
Order: 23-144-73-134-20

[a] Plate crosses were done as described in Materials and Methods. The background overlay consists of the nonpermissive host, DW101, mixed with the underlined amber mutant. For example, plate 23 has a background of P1 *am*23; the spot with the least number of PFU is *am*144; the spots with next lowest PFU are *am*73 and *am*134 which give indistinguishable results, as indicated by parentheses; the *am*20 spot has still more PFU. By comparing all the combinations in reciprocal "crosses" using five test plates, an unambiguous order can be deduced. Mutants 108 and 31 (in adjacent linkage clusters II and IV) give complete clearing within their spots, indicating no detectable linkage (dashed lines) under the conditions of these tests.

PFU, the less the recombination, and, therefore, the closer the mutants to the test mutant in the overlay. This procedure does not give order but only relative distance of mutants from one another. For example, in the sequence 23-144-(73, 134)-20, *am*144 is the mutant closest to *am*23; *am*73 and *am*134 are roughly the same distance away (farther than *am*144 and not as far as *am*20), but they could be: (i) on opposite sides of *am*23; (ii) near each other but on the opposite side to *am*144; or (iii) near each other and on the same side as *am*144. By comparing the 23 pattern with the other four patterns, however, only one unambiguous order results which is consistent with the five patterns.

That unambiguous orders are obtained attests to the validity of this method for determining order of mutants. Supporting evidence for use of the method in this way comes from deletion mapping in linkage cluster IV. The mutants in this cluster were ordered before defective lysogens became available to divide cluster IV into four deletion segments (D, E, F, and G); the results of rescue tests with the four relevant defective lysogens (34) showed no inconsistencies with the order indicated by the spot test method.

As might be expected, ambiguities in linear ordering did occur when mutants were very closely linked. These are indicated on the map (Fig. 1) by parentheses and brackets. When little or no difference between mutants could be discerned either by spot tests or by cross-streaks using 10^9 PFU/ml, the hash marks are placed very close together and the mutants are listed vertically below, indicating that they are in the same cistron. In each instance this was confirmed by complementation tests in broth (see below). Mutants in linkage cluster X behaved as two subgroups (X-1 and X-2); i.e., although mutants in X-1 and X-2 showed some linkage to each other, and no linkage to mutants in any other cluster, there was stronger linkage between mutants within each subgroup than between mutants from the different subgroups. The order of these subgroups and their orientation to each other could not be established by this method, but the order was determined by Scott (29) using rescue tests with the defective prophage P1*d*91*tet*, which rescues $c1^+$ and *am*10.1$^+$ but not *am*9.16$^+$ or *am*10.70$^+$. Extensive two-factor crosses in broth might clarify the order of mutants in parentheses in X-1 and X-2 as well as in several of the other linkage clusters.

Three-factor crosses. Linkage clusters III, V, VII, and VIII could not be added to the P1 map of Scott by two-factor spot tests. Cluster III was located on the map after defective lysogens (34) became available from Scott, Stodolsky, and Rosner. Figure 1 shows that the *am*$^+$ alleles of the mutants in cluster III are rescued by the defective lysogens K140(P1*cry*), ΔCM2, PL0148, and PL0200, but not by 8a (see Walker and Walker [34] for details).

Marker rescue tests with defective lysogens (34) showed that the remaining three clusters (V, VII, and VIII) were located in deletion segment H (see Fig. 1) and therefore must be adjacent to linkage cluster VI on one side or the other. Using *vir* (located in cluster IV) as an outside and unselected marker, three-factor crosses were done using an amber mutant from linkage cluster VI and one from each of linkage clusters V, VII, and VIII. *am*8.13 (cluster IX) was used as an outside marker on the right to confirm the location of clusters VII and VIII. *am*$^+$ progeny phage were scored as clear or turbid, and these crosses (Table 2) were set up in such a way (with *vir* as an outside marker) that clear plaques would be obtained from single or odd numbers of crossovers and turbid plaques from double or even numbers of crossovers, or vice versa. Reciprocal crosses using *am*107 or *am*180 and *am*7.14 with *vir* and crosses of *vir am*107 or *vir am*180 × *am*79 (first set of crosses, Table 2) located linkage cluster V between clusters IV and VI. The second set of crosses in Table 2 located linkage cluster VIII between clusters VI and IX. The third set of crosses located linkage cluster VII between clusters VI and IX also. The last pair of crosses established the order of clusters VII and VIII.

TABLE 2. *Three-factor crosses with vir as the outside (and unselected) marker*

Cross	Total PFU scored	% am^+	% Clear (am^+)
$\frac{vir \quad + \quad am7.14}{+ \quad am107 \quad +}$	3,329	5.5	89
$\frac{vir \quad am107 \quad +}{+ \quad + \quad am7.14}$	2,962	5.8	16
$\frac{vir \quad + \quad am7.14}{+ \quad am180 \quad +}$	3,101	7.9	90
$\frac{vir \quad am180 \quad +}{+ \quad + \quad am7.14}$	4,127	6.0	14
$\frac{vir \quad am107 \quad +}{+ \quad + \quad am79}$	2,488	6.5	31
$\frac{vir \quad am180 \quad +}{+ \quad + \quad am79}$	1,559	15.5	34
Order indicated: *vir*-(*am*107, *am*180)-(*am*79, *am*7.14)			
$\frac{vir \quad + \quad am71}{+ \quad am7.14 \quad +}$	5,911	2.7	72
$\frac{vir \quad am7.14 \quad +}{+ \quad + \quad am71}$	3,895	1.5	38
$\frac{vir \quad + \quad am151}{+ \quad am7.14 \quad +}$	4,182	1.9	72
$\frac{vir \quad am7.14 \quad +}{+ \quad + \quad am151}$	3,521	1.2	37
$\frac{vir \quad + \quad am71}{+ \quad am79 \quad +}$	917	5.2	76
$\frac{vir \quad + \quad am151}{+ \quad am79 \quad +}$	4,646	3.0	63
$\frac{vir \quad am71 \quad +}{+ \quad + \quad am8.13}$	2,265	6.6	30
$\frac{vir \quad am151 \quad +}{+ \quad + \quad am8.13}$	3,437	3.7	29
Order indicated: *vir*-(*am*79, *am*7.14)-(*am*71, *am*151)-*am*8.13			
$\frac{vir \quad + \quad am30}{+ \quad am79 \quad +}$	235	1.9	91
$\frac{vir \quad + \quad am40}{+ \quad am79 \quad +}$	378	1.4	94
$\frac{vir \quad am30 \quad +}{+ \quad + \quad am8.13}$	1,348	6.0	30
$\frac{vir \quad am40 \quad +}{+ \quad + \quad am8.13}$	1,360	4.4	28
Order indicated: *vir*-*am*79-(*am*30, *am*40)-*am*8.13			
$\frac{vir \quad + \quad am151}{+ \quad am78 \quad +}$	1,716	0.8	73
$\frac{vir \quad + \quad am71}{+ \quad am78 \quad +}$	807	0.3	76
Order indicated: *vir*-*am*78-(*am*151, *am*71)			

Order indicated from all crosses: *vir*-(*am*107, *am*180)-(*am*79, *am*7.14)-(*am*78, *am*30, *am*40)-(*am*151, *am*71)-*am*8.13. (Results of broth complementation tests show that *am*78, *am*30, and *am*40 are in the same cistron.)

The orientation of these clusters with respect to mutants in adjacent clusters could not be determined from the crosses.

Complementation tests in broth. Once the order of the mutants was established on the map by the methods indicated above, allocation of the mutants to cistrons was attempted using classical complementation tests in broth. Three criteria were used in deciding whether complementation had occurred in mixed infection with any two mutants: burst size, increase in progeny compared to progeny obtained in single infections with each mutant separately, and percentage of wild-type (am^+) recombinants. Initially, we tested those groups of mutants (containing from two to six mutants) that gave little or no recombination in spot tests (or cross-streaks) on the assumption that they probably would not complement and we could gradually narrow down the potential number of cistrons. Such groups are represented on the map in Fig. 1 as: (i) vertically stacked under very close hash marks, (ii) within parentheses, or (iii) a combination of (i) and (ii), e.g., mutants 173, 57, 76, 176, and 129 in linkage cluster I. In all such cases, no complementation was observed: burst sizes, measured as percentages of a P1*vir* control, were usually ≤ 0.1, and the total number of PFU was generally ≤ 10-fold above background. The proportion of am^+ recombinants was usually <1% in these instances. Exceptions were with some pairs of mutants in the following groups where percentages of am^+ recombinants were 13 to 29%: *am*72, *am*114, and *am*149 in cluster IV; *am*162, *am*2, *am*61, and *am*79 in cluster VI (*am*5 was not tested); and *am*48, *am*58, and *am*131 in cluster X-1. We tentatively conclude that these higher proportions of am^+ recombinants are due to high levels of intracistronic recombination, because low burst sizes and little or no increase in PFU over back-

ground were obtained. Based on these arguments, we would anticipate that with each of these groups the mutants are in the same cistron.

In subsequent complementation tests we used mutants from different putative cistrons within a linkage cluster. In all instances (except *am*7, *am*62, *am*150, and *am*174 in linkage cluster IV [see below]) the proportion of am^+ recombinants was usually <1% and not >4% and did not appreciably alter the amount of *am* progeny obtained. Table 3 shows complementation data of some mutants in linkage cluster I. Those mutants in the same group (4, 128, 132, and 165) gave, unambiguously, no complementation: burst sizes for all six combinations were <0.02 phage/cell, and the total progeny from all combinations was only 0.6 to 2.3× background. Similar results were obtained from combinations 57, 76, 129, 173, and 176 (data not shown). Combinations of mutants in adjacent groups (e.g., *am*129 × *am*165), however, gave a burst size of only ~0.25 (~10× background) and were considered not to complement under the conditions of the test. The outermost marker in linkage cluster I is *am*47. Complementation of *am*47 with *am*129 and *am*165 was considered very weak and therefore was interpreted as ambiguous because the burst sizes, although 5- to 10-fold higher than for *am*129 × *am*165, were only 1 to 2/infected cell, whereas the total progeny were 70 to 140× background. Only *trans* tests were done, and from the data we can tentatively conclude either that all 14 mutants in linkage cluster I belong to only one cistron or that polarity exists. Normal complementation, in terms of burst size, occurs between *am*129 and *am*23, which are in different linkage clusters.

The results of complementation tests with some of the mutants in the F and G deletion segments of linkage cluster IV are shown in Table 4. Mutants 38 and 74 clearly do not complement but with *am*75 give very weak or ambiguous complementation (bursts of 1 to 2 phage/infected cell and increases above background of 350 and 38×). *am*74 gives a similar result with *am*49, but *am*49 with *am*38 and *am*75 gives bursts of 9 and 16, both being significantly above background (450 and >1,000×).

TABLE 3. *Complementation matrix of am mutants in linkage cluster I*[a]

Mutant	129[b]	4	128	132	165	47	23[c]
129	0.04						
4		0.01					
128		0.08 (<0.2)	0.003				
132		0.01 (<0.2)	0.004 (<0.6)	0.003			
165	0.25 (3.0)	0.016 (<0.2)	0.002 (<1.0)	0.008 (<0.3)	0.004		
47	1.97 (3.0)				1.25 (4.0)	0.014	
23	44.4 (7.0)						1.18

[a] Burst sizes are given as percentage of the P1*vir* control (19), which averages 100 to 150 phage/infected cell. The MOI was 5 to 10 for each phage. Percentage of am^+ recombinants (number in parentheses) was calculated by dividing PFU per milliliter obtained on the Su^- host by PFU per milliliter obtained on the Su^+ host and multiplying by 100.

[b] *am*129 and mutants 57, 76, 173, and 176 showed no complementation in all pairwise combinations.

[c] *am*23 is located in linkage cluster III.

TABLE 4. *Complementation matrix of some am mutants in linkage cluster IV*[a,b]

Mutant	49	38	74	75	35
49	0.03				
38	9.12 (1.5)	0.01			
74	1.38 (1.0)	0.07 (0.3)	0.06		
75	15.85 (3.0)	1.95 (1.2)	1.18 (0.2)	0.001	
35			80.45 (0.5)		0.09

[a] See footnote *a* of Table 3.

[b] All five mutants listed under gene 2 in Fig. 1 showed no complementation with each other. However complementation of *am*2.17 with *am*8.13 (cluster IX) gave a burst size of ~30, which was greater than 1,000× background.

These last two results indicate definite but weak complementation. Normal complementation occurs between *am*74 × *am*3.35 where the burst is 80 (~1,000× background).

In linkage cluster II, *am*7, *am*62, *am*150, and *am*174 gave *am*$^+$ recombinant percentages of up to 13% in pairwise combination. These high percentages were considered to be due to high intracistronic recombination because burst sizes were <0.2 (and ≤2× background). These results suggest that these mutants are in the same cistron. *am*117 was not tested but *am*62 × *am*108 gave a burst of ~2 (~270× background) which, like some of the cases in clusters I and IV, may be construed as ambiguous or very weak complementation. The mutants in cluster III have not been tested, nor have those in cluster V. In cluster VI, results were similar to those described for the mutants tested in deletion segments F and G of cluster IV in giving a gradation of normal, weak, ambiguous, and no complementation In cluster VII all five mutants show no complementation. Cluster X-1 shows very weak complementation among mutants of its four subgroups.

Linkage cluster X-2 gave ambiguous results in two different tests with Su$^-$ DW101. Nine of the 10 mutants (*am*10.11 was not included) were tested in pairwise combinations using the *suA* strain DM1013, with the expectation that any existing polarity might be suppressed. This strain, however, not only failed to suppress any polarity, but reduced burst sizes by about 10-fold (from ~2 to ~0.2 between the subgroups and from ~0.1 to ~0.01 within each subgroup). The burst sizes of the background controls were reduced 20 to 1,000×, however, so that if one considers just the increases of "crosses" over background the complementation matrix (not shown) gave patterns consistent with the already established groupings on the map. If all figures could have been multiplied by 100, it would have appeared as if polarity had been suppressed, but the actual result obtained may be due to the relative increase in recombinants appearing among the progeny (as high as 10% *am*$^+$ in one case).

The conclusion from all our complementation experiments is that we are unable to assign mutants to cistrons with any degree of certainty (partially because of obtaining ambiguous results and partially because much more extensive data are needed). Our previous estimate of 50 to 55 cistrons (34) is undoubtedly too high. (Note: We do not feel justified in using previous tentative cistron numbers, the following of which have been published: 34.62 by Scott and co-workers [4, 27-29], Rosner [23], and D'Ari et al. [5]; 35.23, 36.E [E = 73], and 37.20 by Scott and co-workers [4, 27]; 36 by Walker and Walker [35]; and 56.32 and 66.43 by D'Ari et al. [5].)

DISCUSSION

Use of a modification of the conventional complementation spot test enabled us to assign 113 P1 amber mutations to 10 linkage clusters. The fact that, for the most part, unambiguous linear orders could be obtained for the mutations within each linkage cluster using this spot test attests to its usefulness for determining order of mutations for P1. In addition, it provides a fairly rapid method for mapping mutations once they have been assigned to one of the nine deletion segments. This is particularly useful for segment H, which consists of four linkage clusters and contains almost 40% of the amber mutations thus far mapped. The main limitation of the spot test is that when the mutations are very closely linked their order relative to adjacent mutations cannot be determined.

Groups of mutations that could not be ordered in these spot tests were shown to be in the same cistron by complementation broth tests. However, all of the mutants in each linkage cluster have not been tested in all combinations in broth tests to see whether closely linked mutations (e.g., in adjacent groups as determined by results of spot tests) are in the same cistron, the linkage being due to high intracistronic recombination (as shown for *am*7, *am*62, *am*150, and *am*174), or whether they are in adjacent cistrons. Because more extensive data are needed and because results interpreted as ambiguous were obtained from several complementation broth tests, cistron numbers have not yet been assigned to the amber mutations shown on the map in Fig. 1, except for Scott's 10 original designations.

Results of complementation tests in broth were considered to be ambiguous when low burst sizes were obtained even when, with the mixed infection, there was a large increase in PFU over background. The reason for the ambiguities is unknown. It may be that mutants giving such results are in the same cistron. Another possibility is that mutants giving such low burst sizes are affected by polarity. *cis* tests (30) and further testing with *suA* hosts are required to test this possibility. It is also possible that recombination and complementation are connected in some way. Such a connection might be elucidated by determining the proportion of cells giving rise to exclusively *am*$^+$ recombinants, only *am* progeny, or a mixture of *am*$^+$ and *am* phage. The role of recombination

in these broth complementation tests is unclear. Ideally, one would like to examine if and to what extent complementation occurs under conditions where all recombination systems have been blocked; unfortunately, such tests would be difficult to do since the normal burst size of P1 is drastically reduced in both *recA* and *recB* hosts (11).

The phenomenon of linkage clusters may be related only to the spot test procedure, but this seems unlikely because of the high coincidence of deletion prophage termini with regions demarcating the linkage clusters. Of the 14 deletion prophages used for mapping, only five have a terminus that ends within a linkage cluster (see Fig. 1, defective lysogens PL0148, PL0200, 1a, 3a, and 8a). P1*cry* and ΔCM derivatives have a terminus between VIII and IX, and the deletion prophage of PE0414 has a terminus between IV and V. Of the remaining termini, three occur at the end of the vegetative map (between I and X-2 if the genome is represented as a circle), four occur between I and II, and 11 occur between III and IV where *cam*^{r} is inserted in some of the deletion prophages (34). These coincidences of termini and regions demarcating linkage clusters are striking and suggest the possibility of recombination hot spots. Some of these coincidences may simply be due to occurrence of large segments of silent DNA in these regions, or regions for which there are no known mutants, but such segments would be unlikely to account for all of these coincidences.

If recombination hot spots do occur, some type of site-specific recombination may be responsible. The region (III–IV) at which *cam*^{r} is inserted in P1CM (15), and some of the deletion prophages derived from P1CM, is a likely candidate for a recombination hot spot as described previously (34) and may be the location of an insertion sequence as has been suggested by Chesney and Scott (4). In this connection, an inverted repeat of 0.62 kilobases has been found in P1 by Lee et al. (17) but is not at the same location where *cam*^{r} inserts. Naturally occurring *chi* mutations (8, 16) are another possibility for production of recombination hot spots. It is also conceivable that P1's own restriction and modification system may affect recombination in some way. The relative intensity of recombination in these regions is not known but can be tested genetically by performing extensive two- and three-factor crosses among mutants within and between adjacent linkage clusters.

Another factor influencing the occurrence of hot spots could be the interaction of P1 with the *rec* systems of the host, which have been studied by Hertman and Scott (11). They found that recombination is significantly reduced in both *recA* and *recB* hosts. They conclude that vegetative recombination in P1 is promoted primarily by the *recArecB* pathways but that residual recombination does occur. Whether it is due to the *recF* pathway (12) or to a P1-coded pathway is not known.

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