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# Genetic Map of Bacteriophage $\phi$X174

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Bacteriophage $\phi$X174 temperature-sensitive and nonsense mutations in eight cistrons were mapped by using two-, three-, and four-factor genetic crosses. The genetic map is circular with a total length of $24 \times 10^{-4}$ *wt* recombinants per progeny phage. The cistron order is D-E-F-G-H-A-B-C. High negative interference is seen, consistent with a small closed circular deoxyribonucleic acid molecule as a genome.

Bacteriophage $\phi$X174 is the smallest virus known to undergo genetic recombination (14, 15). Observed recombination frequencies range from $10^{-6}$ to $2 \times 10^{-3}$ *wt* recombinants per progeny phage (C. A. Hutchison III, Ph.D. Thesis, California Institute of Technology, Pasadena, 1969). In spite of these low recombination frequencies, genetic analysis similar to that performed with bacteriophages T4 (17) and $\lambda$ (17) has been shown to be possible for $\phi$X174 (C. A. Hutchison III, Ph.D. Thesis) and for the related bacteriophage S13 (1, 18).

To construct a detailed genetic map of $\phi$X174, we examined the results of genetic crosses involving over 100 single-base transition mutants. In this paper, 36 different conditional-lethal mutants in eight cistrons are mapped by using two-, three-, and four-factor genetic crosses. High negative interference (9) is seen, consistent with a constraint for double exchanges imposed by the small circular genome.

A subsequent paper (Benbow et al., *in preparation*) will show that the combined molecular weights of the $\phi$X174 specific proteins coded by these eight cistrons require over 90% of the genome coding capacity. A correspondence exists between cistron size on the genetic map and protein size determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Taken together, these papers show that the genetic map of bacteriophage $\phi$X174 is circular with cistron order and direction of translation D-E-F-G-H-A-B-C.

## MATERIALS AND METHODS

**KC broth.** KC broth (16) was composed of 10 g of tryptone (Difco) and 5 g of KCl, made to 1 liter with distilled water. It was autoclaved for 20 min, and 0.5 ml of 1 M $CaCl_2$ was added after cooling.

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**Denhardt starvation buffer.** This buffer (4) was composed of 5 g of KCl, 1 g of NaCl, 1.2 g of tris-(hydroxymethyl)aminomethane (Tris), and 0.1 g of $MgSO_4$, made to 1 liter with distilled water. The *p*H was adjusted to 8.1 with HCl, the buffer was autoclaved, and 1.0 ml of 1 M $CaCl_2$ was added after cooling.

**Bottom agar.** Bottom agar (16) consisted of 2.5 g of NaCl, 2.5 g of KCl, 10.0 g of agar (Difco), and 10 g of tryptone (Difco), made to 1 liter with distilled water. After autoclaving, 1 ml of 1 M $CaCl_2$ was added to each liter of medium. Twenty-milliliter plates were poured.

**Top agar.** Top agar (16) consisted of 5 g of NaCl, 8 g of agar (Difco), and 10.0 g of tryptone (Difco), made to 1 liter with distilled water. The mixture was autoclaved for 15 min to dissolve the agar, bottled, and then autoclaved again.

**$\phi$X174 nonsense mutants.** *am*N1, *am*H81, and *am*H57 were gifts from M. Hayashi. *am*80, *am*86, *am*87, *am*88, *am*89, *am*90, *och*1, *och*5, *och*6, *och*8, and *och*11 were isolated in this laboratory by F. Funk (7). *cs*70 is the cold-sensitive $\phi$X174 mutant of Dowell (5). All other stocks were grown from single plaques of the original stocks of Hutchison (Ph.D. Thesis). A list of the conditional-lethal mutants used in this study is given in Table 1.

**$\phi$X174 double mutants.** *am*3*ts*$\gamma$, *am*3*ts*79, *am*3*ts*9, *am*3*ts*4, and *am*3*cs*70 were described by Hutchison (Ph.D. Thesis). All other double mutants were isolated from two-factor crosses in which both parental stocks were ultraviolet-irradiated to roughly three lethal "hits" per phage before mating. Screening for double mutants was performed by layering infected, permissive cells in top agar over a prepoured plate containing nonpermissive cells. The plates were incubated at 30 C for 3 hr and then shifted to 40 C for 6 hr. Four plaque types were seen: large-clear, large-turbid, small-clear, and small-turbid. Single plaques of the last type (*am-ts* double mutants) were tested for inability to grow on C (nonpermissive) at 30 C and on HF4714 (permissive) at 40 C; then single plaque isolates were grown to a high titer.

To test the composition of each double mutant, isolates were back-crossed against each of the two parental mutants. If less than $5 \times 10^{-6}$ *wt* recombi-

TABLE 1. *Classification of ϕX mutants*

| Cistron | Mutant |
|---|---|
| A | *am*8, *am*18, *am*30, *am*33, *am*35, *am*50, *am*86, *ts*128 |
| B | *am*14, *am*16, *och*5, *ts*9, *ts*116, *och*1, *och*8, *och*11 |
| C | *och*6 |
| D | *am*10, *am*H81 |
| E | *am*3, *am*6, *am*27 |
| F | *am*87, *am*88, *am*89, *am*H57, *op*6, *op*9, *tsh*6, *ts*41D |
| G | *am*9, *am*32, *ts*γ, *ts*79 |
| H | *am*N1, *am*23, *am*80, *am*90, *ts*4, cs70[a] |

[a] cs70 cannot be assigned to a cistron on the basis of complementation tests (5). We anticipate that it will be found in cistron H, based on its segregation during three- and four-factor crosses and on its physiological characteristics.

nants per total number of progeny phage were found, the single and double mutants were assumed to contain the same mutation. To guard against multiple mutants, only double mutants whose individual markers reverted to wild type at a frequency of $10^{-6}$ to $10^{-7}$ were used. Complementation tests to verify cistron assignments were carried out by A. J. Shafer. All stocks used had titers above $5 \times 10^9$/ml and reversion frequencies below $5 \times 10^{-6}$ *wt* per mutant phage.

**Bacterial strains.** C is the standard nonpermissive host for ϕX174 (16).

HF4704 is a nonpermissive *thy*⁻ host for ϕX174 (11). It is more stringently nonpermissive than C for the *och* mutants used in this study.

HF4714 is permissive for *am* (UAG) but not *och* (UAA) or *op* (UGA) ϕX174 nonsense mutants. HF4714 was isolated in this laboratory by Paul Howard-Flanders. All plating efficiencies are defined relative to ϕX174*wt* on HF4714 at 37 C.

$Su2_{am}$ (7) is an alternative *am* suppressor strain for *am*80 and *am*90, which will not plate on HF4714. Its plating efficiency relative to HF4714 at 37 C is 0.66 for *wt* ϕX174.

$Su2_{och}$ (7) is an *am* and an *och* suppressor strain. Its plating efficiency is 0.66 for *wt* ϕX174.

CIT103 (C. A. Hutchison III, Ph.D. Thesis) is an *op* suppressor strain with a plating efficiency of 1.00 for *wt* ϕX174.

RMB101 is an *am-op* double suppressor strain. It was constructed by lysogenizing CIT103 with ϕ80p $Su_{III}^+$ (donated by J. Parkinson). Its plating efficiency for *am* mutants is generally 1.00 relative to HF4714.

**Growth of stocks.** Single plaques grown at 30 C for 4 to 5 hr were transferred with a sterile pipette to a culture of permissive cells at $10^8$/ml in KC broth. These were gently aerated at 32 C for 2 to 3 hr until the cultures cleared. The lysate was made 0.005 M ethylenediaminetetraacetic acid (EDTA), 0.4 M NaCl, and 6% (w/v) polyethylene glycol 6000. After precipitation in the cold for 1 to 2 hr, lysates were centrifuged at 5,900 × *g* for 20 min. The pellet (or interface, often not visible) was resuspended in 0.05 M sodium tetraborate and centrifuged to remove debris. We thank L. Dumas for suggesting the use of polyethylene glycol 6000.

Very-high-titer stocks of cistron E mutants (except *am*6) were prepared by infecting log-phase HF4704 (nonpermissive) at a multiplicity of infection of 2 and incubating at 32 C for 1.5 hr. Cells were pelleted and artificially lysed with 0.2 mg of lysozyme per ml (in 0.05 M Tris, 0.005 M EDTA, *p*H 8.1) followed by freezing and thawing three times; the phage were collected as above.

High-titer stocks of mutants in other cistrons were obtained by infecting log-phase cultures of permissive cells at a multiplicity of infection of 2. After 20 min, enough 60% sucrose was added to give a final concentration of 12% sucrose (to delay lysis). After 60 min, phage were collected by polyethylene glycol precipitation as above.

**Two-factor genetic crosses (between nonsense mutants).** HF4714 (a permissive host for *am* mutations) was grown to $10^8$ cells/ml at 37 C with gentle aeration. The culture was made 0.003 M KCN and aerated for 10 min. Two 0.5-ml samples of $2 \times 10^8$ ϕX174 mutant phage per ml in KC broth-0.003 M KCN were mixed in a mating tube in an ice bath. A 0.2-ml amount of the KCN-treated bacterial culture was added to each mating tube and incubated at 37 C for 15 min. A 0.2-ml amount of each of the resulting phage-cell complexes was diluted into 20 ml of KC broth. These were vigorously aerated at 36 C for 90 min. Cultures were shaken with chloroform and titered, usually on C and on HF4714 at 37 C. The recombination frequency between *am* mutants was defined as the titer on C at 37 C divided by the titer on HF4714 at 37 C. Control experiments with a 1:1 mixture of parental phage showed that recombination on a plate was always below a frequency of $5 \times 10^{-6}$ *wt* per total progeny phage as was the recombination frequency of a mutant with itself (selfing).

Titers on $Su2_{och}$ were corrected to give HF4714 equivalent titers. Crosses were always performed in HF4714, even when *op* or *och* mutants were used. The titer on RMB101 (an *am op* double suppressor) at 37 C was used directly for *am* × *op* crosses in which the *am* mutants plated with equal efficiency on RMB101 and HF4714.

**Three- and four-factor genetic crosses.** A procedure similar to that for two-factor crosses was employed except that growth was at 32 C. Titers were obtained at both 30 and 40 C (37 and 25 C for *cs*70). To confirm that plating at two temperatures is adequate to determine whether *am* × *am* recombinant progeny from *am*A *ts*C × *am*B crosses are predominantly *wt* or *ts*, a large number of single plaques were grown at 30 C and tested for ability to form plaques at 40 C. The fraction able to do so was similar to that determined from a mass plating at the two temperatures. An analogous control for segregation of the *am* marker in *ts*D *am*F × *ts*E crosses was carried out.

Recombination frequencies are defined as follows: for *am ts* × *am*, as the titer on C at 30 C divided by the titer on HF4714 at 30 C (i.e., *ts* + *wt*/total); for *am ts* × *ts*, as the titer on HF4714 at 40 C divided by the titer on HF4714 at 30 C (*am* + *wt*/total); for

*am ts* × *och*, as the titer on HF4704 at 30 C divided by the corrected titer on $Su2_{och}$ at 30 C (*ts* + *wt*/total); for *am ts* × *op*, as the titer on C at 30 C divided by the titer on RMB101 at 30 C (*ts* + *wt*/total); and for *och am* × *och*, as the titer on HF4714 at 30 C divided by the corrected titer on $Su2_{och}$ at 30 C (*am* + *wt*/total). These are equivalent in all tested cases to the two-factor recombination frequencies.

**Counting statistics.** For each determination of a recombination frequency in Table 2, three plates were counted to measure *wt* recombinants and three were counted for total progeny. Only crosses with burst sizes in excess of 75 under permissive conditions were tabulated. In Table 2, the numbers in parentheses represent the number of independent determinations used to calculate the average recombination frequency. The genetic map in Fig. 1 represents these two-factor crosses and similar unpublished data in a schematic form. No claim is made that this map represents accurately the linear distance between these mutations.

## RESULTS

**Two-factor crosses.** The average frequency of wild-type recombinants produced in pairwise crosses between $\phi$X174 nonsense mutants is shown in Table 2. Several points may be emphasized.

(i) Recombination frequencies between closely linked markers often are roughly additive.

(ii) Recombination frequencies may be ordered to form a circular genetic map, i.e., any marker selected will lie at both ends of a linear map.

(iii) The length of the complete genetic map is $24.4 \pm 3 \times 10^{-4}$ recombination units as calculated by summing the distances between 6(E) - 88(F) - 9(G) - NI(H) - 86(A) - 16(B) - och6(C)-10(D)-6(E). Map contraction is often seen between distant markers.

(iv) *am*3 shows lower recombination frequencies when crossed with certain mutants, such as *am*23, *am*88, and *op*6, than expected from map distances determined with mutants in other cistrons. Most other cistron E mutants (*am*20, *am*24, *am*26, *am*27, *am*29, and *am*34) also show this phenomenon (Benbow, Davis, and Sinsheimer, *in preparation*).

(v) Some cistron A mutants, *am*33 for example, show much higher intracistronic recombination frequencies than anticipated and occasionally higher intercistronic frequencies. Many other cistron A mutants (*am*8, *am*30, *am*81, *am*83, and *am*85) also behave in this manner. This phenomenon will be discussed briefly here and in more detail at a later date (Benbow, Davis, and Sinsheimer, *in preparation*).

(vi) Some mutants, *am*23(H) and *och*6(C) for example, show consistently low recombination frequencies. We believe these represent site-specific recombination effects (Norkin, submitted Ph.D. Thesis, Columbia Univ., New York).

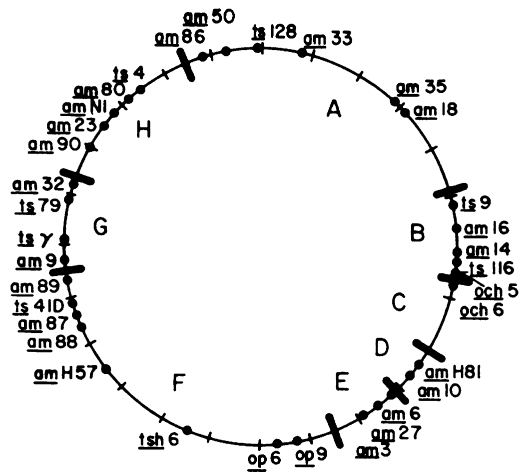


FIG. 1. *Frequency of wt recombinants in two-factor genetic crosses between $\phi$X174 conditional lethal mutants is represented schematically. One map unit represents $10^{-4}$ wt recombinants per total progeny phage, except in the region of cistron A for the reasons noted in the text. Cistron boundaries are arbitrarily drawn. The recombination data for am87, am89, am32, am80, am90, och5, amH81, amH57, am27, and op9 are found in Benbow et al. (in preparation). The order of ts mutations within a cistron relative to the am mutations in the same cistron was determined from three factor crosses (Table 3) and from ts × ts or ts × am two-factor crosses (unpublished data).*

**Three- and four-factor crosses.** The rationale for three-factor crosses is described in Baker and Tessman (1). Briefly, the progeny of a cross of the type *am*A *ts*C × *am*B is plated on a nonpermissive (for *am* mutations) strain at a permissive (for *ts* mutations) temperature (30 C). The *am* × *am* recombinants are then tested at 30 and 40 C to follow segregation of the *ts* marker. If *am*A and *am*B are closely linked and *ts*C is a more distant external marker, then when the progeny are predominantly *ts* the order deduced is *am*A-*am*B-*ts*C. If the progeny are predominantly *wt*, the order deduced is *ts*C-*am*A-*am*B. *am*A and *am*B are considered adjacent if they lie in the same cistron or very closely linked cistrons while the *ts* marker is in a more remote cistron. In most cases, the same result is obtained by plating the progeny from the cross directly at 30 and 40 C on a nonpermissive strain. Analogous reasoning holds for crosses of the type *ts*D *am*E × *ts*F.

An unambiguous order for cistrons A, B, E, F, G, and H is established by the data in Table 3. For essential placements, the reciprocal cross is included since wild-type recombinants occasionally outgrow a predominant mutant geno-

TABLE 2. *Two-factor recombination frequencies*[a]

| Mutant | A<br>*am*18 (A) | *am*33 (A) | *am*35 (A) | *am*50 (A) | *am*86 (A) | B<br>*am*14 (B) | *am*16 (B) | C<br>*och*6 (C) | D<br>*am*10 (D) | E<br>*am*3 (E) | *am*6 (E) | F<br>*am*88 (F) | *op*6 (F) | G<br>*am*9 (G) | H<br>*am*23 (H) | *am*N1 (H) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | | | | | | | | | | | | | | | | |
| *am*18(A) | | | | | | | | | | | | | | | | |
| *am*33(A) | 21.9<br>±3.2<br>(2)[b] | | | | | | | | | | | | | | | |
| *am*35(A) | 0.4<br>±0.1 | 21.2<br>±2.4[b] | | | | | | | | | | | | | | |
| *am*50(A) | 7.9<br>±0.5[b] | 4.5<br>±0.6[b] | 13.9<br>±1.0[b] | | | | | | | | | | | | | |
| *am*86(A) | 11.7<br>±2.4[b] | 5.5<br>±2.0<br>(2)[b] | 16.8<br>±1.5[b] | 0.5<br>±0.1<br>(2) | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | | | | | |
| *am*14(B) | 2.8<br>±0.3 | 11.3<br>±0.4[b] | 2.8<br>±0.3 | 2.6<br>±0.5<br>(2) | 4.0<br>±0.2 | | | | | | | | | | | |
| *am*16(B) | 2.0<br>±0.1 | 9.1<br>±2.8[b] | 2.7<br>±0.1 | 6.4<br>±0.3 | 7.3<br>±1.2 | 1.3<br>±0.3 | | | | | | | | | | |
| **C** | | | | | | | | | | | | | | | | |
| *och*6(C) | 1.3<br>±0.2[c] | 3.9<br>±0.4[c] | 0.7<br>±0.1[c] | 2.2<br>±1.3[c] | 1.1<br>±0.1[c] | 1.0<br>±0.2 | 1.3<br>±0.3 | | | | | | | | | |
| **D** | | | | | | | | | | | | | | | | |
| *am*10(D) | 1.4<br>±0.5 | 6.2<br>±1.0 | 2.0<br>±0.5 | 4.0<br>±0.6<br>(2) | 4.2<br>±0.2 | 1.8<br>±0.2<br>(2) | 2.3<br>±0.4 | 2.0<br>±0.2 | | | | | | | | |
| **E** | | | | | | | | | | | | | | | | |
| *am*3(E) | 4.1<br>±0.6<br>(2) | 10.8<br>±0.8<br>(3) | 4.2<br>±1.0<br>(3) | 10.2<br>±1.6<br>(3) | 8.3<br>±0.9<br>(2) | 3.4<br>±0.5<br>(4) | 4.6<br>±0.9<br>(5) | 1.3<br>±0.1<br>(2)[c] | 1.5<br>±0.2<br>(3) | | | | | | | |
| *am*6(E) | 6.6<br>±1.0 | 5.7<br>±0.8 | 6.3<br>±1.2 | 8.3<br>±2.0 | 6.5<br>±2.0<br>(2) | 2.4<br>±0.7 | 3.5<br>±0.4 | 0.2<br>±0.7[c] | 0.2<br>±0.4 | 0.2<br>±0.4<br>(6) | | | | | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **F** | | | | | | | | | | | | | | | |
| *am*88(F) | 10.8<br>±1.2 | 12.4<br>±2.1 | 11.9<br>±0.3 | 8.0<br>±1.0 | 5.3<br>±0.9 | 10.3<br>±0.7 | 9.3<br>±3.2 | 11.1<br>±2.2 | 14.4<br>±0.6 | 4.4<br>±0.8<br>(2)[c] | 7.1<br>±0.7<br>(2) | | | | |
| *op*6(F) | 6.5<br>±0.2 | Low burst | 6.0<br>±0.2 | 4.2<br>±1.5[c] | 1.3<br>±0.6[c] | 4.8<br>±0.4 | 5.3<br>±0.7 | Low burst | 2.2<br>±0.1 | 1.2<br>±0.1 | 2.5<br>±0.4 | Low burst | | | |
| **G** | | | | | | | | | | | | | | | |
| *am*9(G) | 5.8<br>±1.4<br>(2) | 11.5<br>±0.8 | 8.0<br>±1.0<br>(2) | 8.2<br>±0.8 | 6.8<br>±0.4 | 2.9<br>±0.9 | 5.4<br>±1.2 | 1.2<br>±0.1[c] | 5.9<br>±2.1 | 6.8<br>±0.8<br>(9) | 4.7<br>±1.0 | 1.3<br>±0.2 | Low burst | | |
| **H** | | | | | | | | | | | | | | | |
| *am*23(H) | 1.7<br>±0.8<br>(2)[c] | 2.0<br>±0.4[c] | 4.7<br>±0.5[c] | 1.2<br>±0.1[c] | 0.4<br>±0.1[c] | 1.8<br>±0.5<br>(3)[c] | 2.1<br>±0.4<br>(2)[c] | Low burst | 2.6<br>±0.3[c] | 2.2<br>±0.6<br>(2)[c] | 3.4<br>±0.9[c] | 3.4<br>±0.4 | 9.2<br>±1.4 | 2.1<br>±0.3[c] | |
| *am*N1(H) | 3.0<br>±0.3 | 7.5<br>±1.2 | 3.1<br>±0.2 | 2.0<br>±0.3<br>(2) | 2.1<br>±0.3<br>(2) | 3.0<br>±0.5 | 2.8<br>±0.5 | 1.4<br>±0.2[c] | 4.6<br>±0.3<br>(2) | 8.1<br>±1.3<br>(3) | 6.2<br>±0.9<br>(2) | 4.1<br>±0.6 | 8.1<br>±0.5 | 3.1<br>±0.8<br>(2) | 0.26<br>±0.3<br>(2) |

[a] Results expressed × $10^4$.

[b] These cistron A recombination frequencies were used in the construction of Fig. 2 only. Numbers in parentheses represent the number of independent determinations used to calculate the average recombination frequency.

[c] Exact correspondence between map distance in Fig. 1 or 2 and recombination frequency is not obtained for the reason noted in the text.

TABLE 3. *Three-factor recombination frequencies*

| Cross | Recombination frequency[a] | Per cent *wt* | Predominant genotype | Order deduced or confirmed (H G F E D C B A H G) |
|---|---|---|---|---|
| (1) *am*N1 *ts*γ × *ts*79 | 1.2 ± 0.4 | 33 | *am* | N1—79—γ |
| (2) *am*N1*ts*79 × *ts*γ | 0.8 ± 0.3 | 90 | *wt* | N1—79—γ |
| (3) *am*88*ts*γ × *ts*79 | 1.0 ± 0.7 | 97 | *wt* | 79—γ-88 |
| (4) *am*88*ts*79 × *ts*4 | 1.8 ± 0.4 | 96 | *wt* | 4-79—88 |
| (5) *am*88*ts*79 × *op*6 | 13.4 ± 3.7 | 91 | *wt* | 79—88—6 |
| (6) *am*88*ts*79 × *am*9[b] | 1.4 ± 0.3 | 38 | *ts* | 79—9-88 |
| (7) *am*3*ts*79 × *ts*γ | 0.7 ± 0.1 | 25 | *am* | 79—γ—3 |
| (8) *am*3*ts*γ × *ts*79 | 0.9 ± 0.2 | 88 | *wt* | 79—γ—3 |
| (9) *am*88*ts*79 × *am*3 | 3.0 ± 0.3 | 99 | *wt* | 79—88—3 |
| (10) *am*3*ts*γ × *am*88 | 4.3 ± 0.5 | 46 | *ts* | γ-88—3 |
| (11) *am*3*ts*γ × *op*6 | 1.2 ± 0.1 | 43 | *ts* | γ—6-3 |
| (12) *am*3*ts*γ × *am*27 | 0.3 ± 0.2 | 95 | *wt* | γ—3-27 |
| (13) *am*3*ts*9 × *am*27 | 0.3 ± 0.2 | 37 | *ts* | 3-27—9 |
| (14) *am*3*ts*9 × *ts*116 | 2.3 ± 0.4 | 16 | *am* | 3—116—9 |
| (15) *am*33*ts*116 × *ts*9 | 1.5 ± 0.3 | 39 | *am* | 116—9-33 |
| (16) *am*33*ts*116 × *am*50 | 4.5 ± 0.6 | 89 | *wt* | 116—33-50 |
| (17) *am*33*ts*116 × *am*86 | 7.1 ± 2.2 | 77 | *wt* | 116—33-86 |
| (18) *am*33*ts*4 × *am*16 | 9.7 ± 1.5 | 95 | *wt* | 16-33—4 |
| (19) *am*33*ts*79 × *ts*γ | 1.0 ± 0.4 | 79 | *wt* | 33—79—γ |
| (20) *am*33*ts*γ × *am*N1 | 8.3 ± 1.1 | 34 | *ts* | 33—N1—γ |
| (21) *am*9*ts*128 × *am*N1 | 3.0 ± 0.3 | 7 | *ts* | 128—N1—9 |
| (22) *am*33*ts*γ × *am*86 | 6.0 ± 0.8 | 43 | *ts* | 33-86—γ |
| (23) *am*9*ts*128 × *am*88 | 1.6 ± 0.3 | 99 | *wt* | 128—9—88 |
| (24) *am*88*ts*79 × *och*6 | 10.8 ± 2.3 | 93 | *wt* | 79—88—6 |
| (25) *am*3*ts*γ × *och*6 | 1.4 ± 0.3 | 95 | *wt* | γ—3—6 |
| (26) *am*3*ts*9 × *och*6 | 1.0 ± 0.4 | 45 | *ts* | 3—6—9 |
| (27) *am*3*och*8 × *och*6 | Low burst | 4 | *am* | 3—6—8 |
| (28) *am*3*och*11 × *och*6 | Low burst | 6 | *am* | 3—6—11 |
| (29) *am*3*och*6 × *och*1 | Low burst | 95 | *wt* | 3—6—1 |
| (30) *am*3*och*6 × *och*5 | Low burst | 98 | *wt* | 3—6—5 |
| (31) *am*33*ts*116 × *och*6 | 3.9 ± 0.4 | 21 | *ts* | 6—116—33 |
| (32) *am*33*ts*4 × *och*6 | 3.0 ± 1.2 | 97 | *wt* | 6—33—4 |

| Cross | | | | |
|---|---|---|---|---|
| (33) *am*88*ts*79 × *am*10 | 14.0 ± 0.5 | 99 | *wt* | 79——88——————10 |
| (34) *am*3*ts*79 × *am*10 | 1.4 ± 0.7 | 90 | *wt* | 79——————3———10 |
| (35) *am*3*ts*γ × *am*10 | 1.3 ± 0.2 | 99 | *wt* | γ————3———10 |
| (36) *am*3*ts*9 × *am*10 | 1.6 ± 0.1 | 42 | *ts* | 3———10——————9 |
| (37) *am*33*ts*116 × *am*35 | 18.4 ± 1.2 | 55 | Intermediate | 116——(35, 33) |
| (38) *am*33*ts*116 × *am*18 | 21.9 ± 6.8 | 56 | Intermediate | 116——(18, 33) |

[a] Values expressed × $10^4$.

[b] This cross demonstrates that *ts*79 segregants are not outgrown by *wt* segregants in crosses 4, 5, 7, and 33.

type. [This distortion was also seen by Baker and Tessman (1).]

The genotype which is not predominant is always present in at least 5% of the recombinant progeny. This high negative interference is shown in Table 4 by selecting single *am* × *am* recombinant plaques at 30 C and testing for segregation of *ts* marker by plating at both 30 and 40 C. Four-factor crosses of the type *am-ts* × *am-cs*, selecting for recombination between the *am* markers, confirm this by showing that all four of the recombinant genotypes thus selected, ++-++, +++*cs*, *ts*+++, and *ts*++*cs*, are generated in significant amounts.

The placement of cistron C is more difficult since only a single *och* mutant site has been isolated, i.e., three independent *och* isolates complementing in cistron C show no recombination ($5 \times 10^{-6}$) among themselves. The two-factor data in Table 2 are ambiguous; *och*6 generally exhibits low recombination frequencies. Three-factor crosses in Table 3 appear to place *och*6 unequivocally. However, the crucial crosses, 27, 28, 29, and 30, are of the type *och am* × *och* for which we have no suitable controls (i.e., crosses with *och* mutants in cistrons of known location). With this reservation, cistron C has a clearly defined map position between cistron B and cistron E.

Cistron D is also placed between cistrons B and E by the three-factor crosses in Table 3. Unfortunately, reciprocal crosses of the type *am ts* × *ts* or *am ts* × *am* cannot be carried out because our nonsense mutants in cistron D are temperature-sensitive when grown in our suppressor-carrying hosts. The placement of cistron D relative to cistron C depends on the fact that the cross *am*33*ts*116 × *am*10 segregates a lower percentage (25%) of *ts* recombinants on a nonpermissive strain than does the cross *am*33*ts*116 × *och*6 (79%). This strongly indicates that *och*6 and *ts*116 are very closely linked, whereas *am*10 and *ts*116 are more widely separated markers.

We would like to note that the location of the cistron A mutants, *am*18 and *am*35, relative to *am*33 is uncertain (Table 3; see below for discussion). With these two exceptions, the three-factor cross data in Table 3 provide our strongest self-consistent evidence for the ordering of the eight known cistrons of φX174.

## DISCUSSION

Two-factor recombination frequencies provide a consistent marker order in many cases. Exceptions generally involve two or three closely linked markers widely separated from neighboring markers, i.e., the set *am*10, *am*3, and *am*6 or the pair *am*14 and *am*16, for example.

The low recombination frequencies found with most cistron E mutants in certain crosses arise because single bursts in these crosses are exceptionally large and asymmetric even under permissive growth conditions. An allelic ratio of 4:1 or more in favor of the cistron E mutant is obtained (Benbow, Davis, and Sinsheimer, *in preparation*). The low recombination frequency is thus an artifact of our method of calculating the recombination frequency by dividing the *wt* progeny by the total progeny since cistron E mutants can rapidly outgrow many partially supressed *am* mutants.

In contrast to the widespread occurrence of *am* and *op* (*unpublished data*) nonsense mutations, *och* mutants have been located in only two cistrons (B and C). It is of interest that these *och* mutations (the presumed *in vivo* termination codons) are found only in the last two cistrons to undergo translation (Benbow et al., *in preparation*).

The high recombination region found within cistron A was observed by Hutchison (Ph.D. Thesis) and also by Tessman (18) in cistron IV of bacteriophage S13. (See Table 5 for an com-

TABLE 4. *Illustration of high negative interference*

| Type of cross | Cross | *ts* | *wt* | Per cent wt | |
|---|---|---|---|---|---|
| | | | | Single colonies | Mass plating |
| Three factor | *am*9*ts*128 × *am*N1 | 107 | 5 | 4.5 | 7 |
| | *am*88*ts*79 × *am*9 | 65 | 35 | 35 | 38 |
| | *am*88*ts*79 × *am*3 | 14 | 116 | 92 | 91 |
| | | *cs*++*ts* | +++*ts* | *cs*+++ | ++++ |
| Four factor | *am*3*cs*70 × *am*88*ts*79 | 23 | 19 | 136 | 43 |
| | *am*3*cs*70 × *am*N1*ts*. | 41 | 42 | 132 | 12 |

TABLE 5. *Cistrons of bacteriophages $\phi$X174 and S13*[a]

| New designation | Previous designations | | |
|---|---|---|---|
| | $\phi$X174 (Sinsheimer) | $\phi$X174 (Hayashi) | S13 (Tessman) |
| A | VI | C | IV |
| B | IV | B | II |
| C | VIII | H(?) | VI(?) |
| D | V | D | VII |
| E | I | G | V |
| F | VII | E | I |
| G | III | F | IIIa |
| H | II | A | IIIb |
| I | | I | |

[a] Above nomenclature and correspondence of cistrons were agreed upon at the small deoxyribonucleic acid phage conference held at the California Institute of Technology on 7 November 1970.

parison of $\phi$X174 and S13 cistrons from various laboratories.) It may arise because one strand frequently is nicked in this region during replication. Alternatively, these mutants may remain longer on the membrane or be exposed to some other physiological circumstance such as an altered deoxyribonucleic acid configuration that stimulates recombination. In this context, we would like to note that, although we have drawn our map as circular in Fig. 1, the data in Table 2 can be represented as shown in Fig. 2, after making the assumption that exchanges *within cistron* A usually are paired. The physical significance of this is unknown at present.

The order of $\phi$X174 cistrons agrees with the order of Baker and Tessman (1) of six homologous S13 genes [using the homologies determined by the complementation tests of Hutchison (Ph.D. Thesis)]. S13 cistron VI, which previously was mapped between our $\phi$X174 cistrons E and F (1), now corresponds to our position for cistron C (I. Tessman, *personal communication*). The existence of a ninth complementation group has also been reported (M. Hayashi, *personal communication*). The general features of the $\phi$X174 genetic map, its additivity, circularity, and high negative interference, are similar to those previously established for the related bacteriophage S13 (1, 18).

The occurrence of high negative interference (1, 9) is indicated by our three-factor genetic cross data. Even for a closely linked outside marker, both external genotypes are found in significant percentages after a recombination event. This is confirmed by the four-factor crosses in Table 4 in which all four possible genotypes are found in significant amounts after selection for recombination between two markers. This makes sense if genetic recombination of $\phi$X174 involves hybrid regions of 500 or more nucleotides on either side of a duplex breakage and reunion region (13). Since there are only 5,500 nucleotides in the entire $\phi$X174 genome, gene conversion by deoxyribonucleic acid repair should occur over a significant portion of the genetic map resulting in the high negative interference observed.

The sum of the shortest distances between two mutations often adds up to more than the measured value, although the order of the markers is usually unambiguous. This "map contraction" may be related to the phenomenon of "map expansion" shown by Holliday (10). Alternatively,

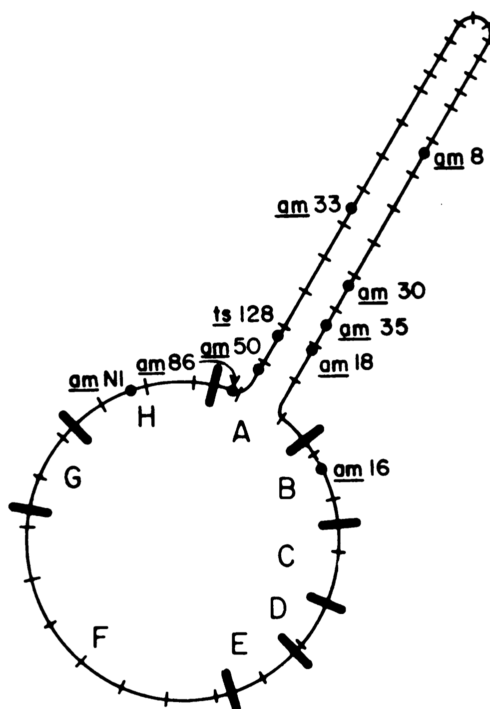


FIG. 2. *Frequency of wt recombinants in two-factor genetic crosses between $\phi$X174 conditional lethal nonsense mutants taken from Table 2. This map is identical to Fig. 1, except that the cistron A region has been drawn to scale to illustrate the very high recombination frequencies found for certain cistron A mutants. To do this, the critical assumption made is that cistrons H and B are closely linked, i.e., that recombination events within the cistron A "loop" usually occur in pairs so that the cistron A region is inert genetically with respect to the rest of the genome. The similarity between this diagram and the previously suggested structure for an exonuclease I-resistant deoxyribonucleic acid fragment of $\phi$X174 (6) is striking.*

it may result from the physical constraints of a small circular genome. We do not know the effect, if any, this has on three-factor crosses.

In concluding we would like to point out that bacteriophage φX174 provides a unique opportunity for genetic analysis in that a fine structure map (2) of the entire genome may be constructed. Unlike the case for most other viruses, single-burst experiments measuring production both of recombinants and of total progeny phage provide the equivalent of single tetrad analysis in fungi (10) since recombination events are very infrequent. Furthermore, most, if not all, of the cistron products of φX174 are well characterized (8, 3, 12), and, therefore, physical verification of the genetic analysis is easily obtained.


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