A DISCREPANCY BETWEEN GENETIC AND PHYSICAL LENGTHS ON THE CHROMOSOME OF BACTERIOPHAGE LAMBDA¹

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A genetic map is an arrangement of genes in which the distance between any pair of genes is proportional to the average number of exchanges between them. Such a map will correspond to the actual distribution of genes on a chromosome only if the probability of exchange and recovery of recombinants is uniform along the chromosome. It was the purpose of the present investigation to detect any extensive departure from uniformity in the probability of exchange or recovery along the chromosome of bacteriophage λ .

It has been shown that recombination in bacteriophage λ occurs by breakage and joining of the double-stranded DNA molecule which composes the phage chromosome (MESELSON and WEIGLE 1961; KELLENBERGER, ZICHICHI and WEIGLE 1961; IHLER and MESELSON 1963; MESELSON 1964). Unexpected features of the results of MESELSON and WEIGLE (1961) led to this research. It was noticed that the distribution of parental DNA among progeny phages of certain genotypes was not as expected from random breakage of the chromosome during recombination. The results reported here elaborate and help to clarify those observations.

Crosses were carried out between heavy phages, labeled with $C^{13}N^{15}$, and light phages. One of the parents was marked at or near one end of the chromosome. The density distribution of the phages with the marker originally in the light parent was then observed in a $CsCl$ density gradient. The results suggest that the production of phages recombinant in a certain region of the chromosome is greatly depressed. In addition, the left end of the λ chromosome has been identified as close to the marker sus_{11} , the farthest known marker on that side of the genetic map. This, in conjunction with the identification of the right end of the chromosome near mi (MESELSON and WEIGLE 1961), means that essentially the whole λ chromosome is included in the known genetic map.

MATERIALS AND METHODS

Escherichia coli strains. Strain C600 (APPLEYARD 1954) was used for the preparation of all phage stocks. Strain C (BERTANI and WEIGLE 1953) was employed as host for crosses involving host-induced modification; for other crosses strain C600 or W3110 (E. LEDERBERG) was used. As indicator strains, in addition to C600 and C, strain W3350 Str^R (obtained from A. CAMPBELL) and C600.4, a host-modification mutant derived from C600, were used. The ability of various

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TABLE 1

Properties of indicator strains

<i>E. coli</i> strain	Host-induced modification type	Ability to plate phages		
		λ -C	λ -K	λ_{sus}
C600.4	C	+	+	+
C	C	+	+	—
C600	K	—	+	+
W3350 Str ^R	K	—	+	—

phages to plate on these strains is shown in Table 1. Following ARBER and DUSOIX (1962), λ able to plate on K or C is designated as λ -K, λ able to plate only on C as λ -C. The plating efficiency of λ -C on K strains varies between 10^{-3} and 10^{-4} .

Bacteriophage strains. Phage λ is the "wild type" of KAISER (1957). The suppressor-sensitive (*sus*) strains are described by CAMPBELL (1961). We have also used the minute plaque marker *mi* (KAISER 1955) and the clear plaque marker *c*₂₆ (MESELSON 1964). All these phages have buoyant density 1.508 g cm^{-3} in pure CsCl solution at 20°C , pH 7.4.

Preparation of phage stocks. Stocks of light phages were prepared by scraping plates (ADAMS 1959). Heavy phages were prepared as described by MESELSON and WEIGLE (1961): λ , $\lambda_{sus_{54}}$, $\lambda_{sus_{73}}$, $\lambda_{sus_{11}}$, by induction of C600 lysogenic for the respective phages, λ_{sus_3} by infection of C600. After the addition of 0.01 percent gelatin to stabilize the phages, the lysates were dialyzed against suspension medium (WEIGLE, MESELSON and PAIGEN 1959). The density homogeneity of the stocks was checked in a CsCl density gradient. The plating efficiency of the λ_{sus} stocks on W3350 Str^R ranged between 6×10^{-4} and 4×10^{-6} .

Density-gradient centrifugation. The technique described by MESELSON and WEIGLE (1961) was employed. Centrifugation was at 23,000 rev/min for 20 to 24 hours at 20°C .

Crosses. An overnight culture of strain C600 or strain C was grown to a concentration of 2×10^9 cells/ml, centrifuged, and resuspended in 0.01 M MgSO_4 . Of these cells, 2×10^8 were infected with a multiplicity of 3 to 5 of each parent phage. After filtration to remove unadsorbed phages, the cells were resuspended in tryptone broth at 5×10^7 cells/ml and grown for 100 min, at which time they were lysed with chloroform. The lysate was freed of bacterial debris by centrifugation and mixed with a saturated solution of CsCl (American Potash and Chemical Corporation) to give an index of refraction $\eta_D^{25} = 1.3805$.

RESULTS AND DISCUSSION

The results expected for uniform probability of exchange along the λ chromosome are illustrated in Figure 1. The figure shows the density distribution of phages which have picked up the marker *x*, originally present on the light parent in a cross between light and heavy phages. The distribution of such phages was calculated by adding the contributions made by recombinants arising from a single exchange at each of 20 equally spaced points on the chromosome. For simplicity, only single exchanges were considered. The shape of the individual distributions was taken from a typical gradient curve. The lower series of distributions represents recombinant phages produced by exchanges occurring between a heavy chromosome and a light one. The middle series represents recombinants produced by exchanges between hybrid and light chromosomes plus the products of replication from the lower series. The highest peak represents all phages with

FIGURE 1.—Illustration of the expected density distribution of phages carrying the marker x which was originally present in the light parent of a cross between light and heavy phages. For details see text. The arrows indicate the points chosen for measuring the position of shoulders for Table 2. They are the points directly over the peak of the first distribution of recombinants contributing to the shoulder. (a) Curve expected if breakage is equally likely at any point. (b) Curve showing the effect of greatly lowered or zero probability of recombination in a region near x , including 14 percent of the chromosome.

light chromosomes. Figure 1a shows the distribution of phages carrying the marker x from the light parent if recombination is equally probable at each of the selected 20 points on the chromosome. Figure 1b shows the effect on the shape of this distribution if recombination is much less probable or absent in one region of the chromosome. Notice that the chromosome can be surveyed only about one third of the way in from the marked end due to limited resolution in the density gradient.

A map of the markers used in these experiments is shown in Figure 2. Crosses involving markers at the left end of the chromosome yielded results closely similar

FIGURE 2.—A map of λ showing the markers under consideration.

to those expected for a uniform probability of recombination. A cross with the marker sus_{11} , the leftmost known marker on the λ map, is illustrated in Figure 3. The distribution of sus^+ phages extends almost as far as the peak of conserved, heavy phages, indicating that sus_{11} is within a few percent of the end of the chromosome. There is no evidence for departure from uniform recombination, that is the curve corresponds fairly well to that shown in Figure 1a. In several subsequent crosses, host-induced modification (see LURIA 1962) was exploited to suppress the appearance of the peak of light phages. It was hoped this would improve the resolution of the shoulders, especially the second shoulder which is barely distinguishable in Figure 3. ARBER and DUSOIX (1962) have shown that the host-specificity of phage λ is a property of its DNA but is not hereditary, being a function solely of the host in which the phage DNA is synthesized. We have employed the K-C system of host-induced modification. *E. coli* strain C accepts phages previously grown in either strain K or C, but K accepts only phages having at least one strand of DNA synthesized in K. We therefore carried out several crosses in strain C with phages prepared on strain K. By plating on strain K, the peak of light phages is strongly suppressed. Unfortunately, the use of host-induced modification seems to improve the resolution of the shoulders only slightly, if at all.

Figure 4 illustrates the result of a cross involving the marker m_6 and using host-induced modification. The distribution of λm_6 shows that m_6 is close to the end of the chromosome, though not so close as sus_{11} . Again, there is no evidence for nonuniform exchange at this end of the chromosome.

Crosses with markers near the right end of the λ chromosome yielded radically different results. Figure 5 shows a cross involving mi , the marker nearest to this end. No host-induced modification is involved in the cross. The distribution of mi recombinants has an initial peak followed by a dip instead of the expected level shoulder. A similar dip occurs in the combined distribution of all mi types from the cross $\lambda c mi \times \lambda C^{13}N^{15}$ and in the combined distribution of all mi^+ types from the cross $\lambda \times \lambda c mi C^{13}N^{15}$ described by MESELSON and WEIGLE (1961).

In order to define more precisely the chromosomal location of the discrepancy observed in Figure 5, crosses were performed with markers proximal to mi . Figure 6 shows a cross involving both mi and sus_{54} , a marker which is close to mi on the genetic map. Two distributions of recombinants are depicted in the figure: one for those phages that have picked up mi and one for those that have picked up sus_{54}^+ . Both curves show the peak and dip already observed in Figure 5. Host-induced modification was employed in this cross, and a comparison of Figures 5 and 6 will show that its use does not significantly affect the features with which we are concerned. In a cross with sus_{73} , shown in Figure 7, the peak

FIGURE 4.— λ -KC C¹³N¹⁵ $\times$ λm_6 K crossed in strain C. Titer of progeny phages in drops collected after centrifugation. ● — $\lambda m_6 + K$; ○ — λm_6 ; K. — λ .

FIGURE 3.— λ SUS₁₁ C¹³N¹⁵ $\times$ λ crossed in strain C600. Titer of progeny phages in drops collected after centrifugation. ● — λ ; ○ — λ SUS⁺; — λ SUS.

FIGURE 6.— λ_{sus+K} $C^{13}N^{15}$ $\times$ $\lambda_{mi}K$ crossed in strain C. Titer of progeny phages in drops collected after centrifugation. ●—● $\lambda_{mi}+K$; ○—○ Total $\lambda_{mi}K$; ▲—▲ Total $\lambda_{sus}+K$.

FIGURE 5.— λ $C^{13}N^{15}$ $\times$ λ_{mi} crossed in strain W3110. Titer of progeny phages in drops collected after centrifugation. ●—● $\lambda_{mi}+$; ○—○ λ_{mi} .

FIGURE 7.— $\lambda_{sus73}K C^{13}N^{15} \times \lambda K$ crossed in strain C. titer of progeny phages in drops collected after centrifugation. ● — Total λK ; ○ — Total $\lambda_{sus} + K$; ○ — Total λK .

FIGURE 8.— $\lambda_{sus3}K C^{13}N^{15} \times \lambda miK$ crossed in strain C. titer of progeny phages in drops collected after centrifugation. ● — Total $\lambda mi + K$; ○ — Total $\lambda_{sus} + K$; ▲ — Total $\lambda mi K$.

and dip are still present, but in a cross with sus_3 , shown in Figure 8, there is no longer any evidence of a dip. The marker mi is included in the cross shown in Figure 8 for comparison. These crosses were all performed with and without host-induced modification with essentially the same results.

Comparison of the curves in Figures 5–8 with the genetic map shown in Figure 2 will reveal that crosses with all markers to the right of sus_3 give anomalous results, while crosses with sus_3 itself do not. The anomalous behaviour here observed therefore begins between sus_{73} and sus_3 .

Two hypotheses could be proposed to explain the shape of the anomalous curves. The peak may be produced by an excess of recombinants in the region beginning between sus_3 and sus_{73} and extending possibly to the end of the chromosome. Alternatively, there may be a deficiency of recombinants between sus_3 and sus_{73} , resulting in the dip. This possibility is illustrated in Figure 1b. A deficiency of recombinants could be produced either by a low probability of exchange or by a lowered recovery of infectious phages.

The possibility of a lowered recovery of infectious phages in the dip region was explored further. It is possible that phages in this region of the gradient are non-infectious. Suppose that genetic recombination produces a damage, say an interrupted strand, in the region where breakage and joining occurs and that the damaged chromosomes are often matured without undergoing repair. If the damage is located in a vital early cistron such phages may be noninfectious. However, they might be revived and thereby detected by complementation with helper phages able to supply the vital function. Experiments to test this possibility were carried out using as helper phages λi^{434} , λ with a sus mutation in a distant part of the chromosome, or a late defective λ . All these experiments were entirely negative. The possibility that there are defective phages in the dip region is thus made unlikely, although not ruled out.

We have been able to obtain additional evidence for a region with anomalous recombination which suggests that the anomaly results from a deficiency of phages recombinant between sus_3 and sus_{73} . It is possible to construct a rough physical map of the chromosome of λ using the data of Figures 3–8 and similar experiments, by measuring the position of the shoulders in the density distributions of recombinants. The legend to Figure 1 explains how the points used in making the measurements were chosen. In Table 2 are given the results of such measurements. The second column gives the distance from the first shoulder to the peak of light phages as a fraction of the distance between light and heavy peaks. The third column gives the same fraction for the second shoulder. In the fourth and fifth columns these fractions are converted to distances on a physical map. Table 3 shows various physical map distances in comparison with the corresponding genetic map distances obtained by AMATI and MESELSON (1965).

The tabulated ratios of physical to genetic length are not at all precise. Our estimates of the physical lengths of the shorter intervals are subject to experimental uncertainties of the magnitude of the estimates themselves. Therefore, the differences between the various ratios cannot be considered significant, except in the case of interval 3. The calculated ratio of physical to genetic length for

TABLE 2
Calculation of physical map distances

Marker	1st shoulder	2nd shoulder	Average (column 2) + (2 $\times$ column 3)	Distance from end of chromosome (%)
<i>mi</i>	0.96 0.94 0.91 0.94	0.49 0.47 0.49 0.53	0.96	4
<i>sus₅₄</i>	0.97 0.92 0.95 0.96	0.46 0.49 0.46 0.48	0.95	5
<i>sus₇₃</i>	0.96 9.92 0.92	0.49 0.44 ...	0.93	7
<i>sus₃</i>	0.57 0.57 0.69	0.32 0.35 ...	0.64	36
<i>m₆</i>	0.89	0.47	0.92	8
<i>sus₁₁</i>	0.96	0.48	0.96	4
<i>C₂₆</i>	0.62	...	0.62	38

For details see text.

this interval is much greater than any other, including that for the very long interval *sus₁₁-mi*. We therefore conclude that the production of phages recombinant in the interval *sus₃-sus₇₃* is greatly depressed.

SUMMARY

Crosses have been performed between isotopically heavy and light phages. The distribution of heavy DNA among phages with markers picked up from the light parent was determined in a density gradient. By this means a rough physical map

TABLE 3
Comparison of physical and genetic lengths

Interval	Genetic length		Physical length (% of chromosome)	Ratio (physical length/ genetic length)
	Observed (% recom- bination)	Corrected for interference* (centi- morgans)		
(1) <i>sus₅₄ — mi</i>	0.5	1.2	1	0.8
(2) <i>sus₇₃ — sus₅₄</i>	1.0	3.0	2	0.7
(3) <i>sus₃ — sus₇₃</i>	1.4	4.7	29	6.0
(4) <i>c — sus₃</i>	1.1	3.4	2	0.6
(5) <i>m₆ — sus₁₁</i>	1.6	5.6	4	0.7
(6) <i>sus₁₁ — mi</i>	...	70*	92	1.3

* See ANATI and MESELSON (1965).

has been constructed. The left end of λ has been identified as near the marker *sus*₁₁. The right end of the chromosome had previously been identified as near *mi*. The production of phages recombinant in a certain region of the λ chromosome appears to be greatly depressed.

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