GENETICS OF THE LEFT ARM OF THE CHROMOSOME OF BACTERIOPHAGE LAMBDA[1]

JOHN S. PARKINSON[2]

Division of Biology, California Institute of Technology, Pasadena, California

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THE chromosome of temperate bacteriophage lambda consists of three functionally distinct regions of approximately equal size. The right arm contains the genes for regulation (EISEN *et al.* 1966), DNA synthesis (JOYNER *et al.* 1966) and cell lysis (HARRIS *et al.* 1967). The central portion of the λ DNA molecule is concerned with the processes of prophage integration (KELLENBERGER, ZICHICHI and WEIGLE 1961; ZISSLER 1967) and vegetative recombination (FRANKLIN 1967). Neither of these latter functions is essential for vegetative growth and the DNA in this region can be deleted with no loss of viability (FRANKLIN 1967; HUSKEY 1968). The left arm of the lambda chromosome is comprised of genes controlling DNA maturation (DOVE 1966; SALZMAN and WEISSBACH 1967) and genes which code for the structural components of the lambda phage particle (WEIGLE 1966).

Although many studies have been devoted to the right and central portions of the lambda chromosome, the little that we know about the left arm is mostly of a qualitative nature. CAMPBELL (1961) isolated suppressor-sensitive (*sus*) nonsense mutants of λ and defined 13 complementation classes in the left arm on the basis of complementation spot tests. He also ordered these 13 classes with respect to one another by deletion mapping with λdg transducing phage. The present study was prompted by this lack of quantitative genetic information concerning the left arm of the lambda chromosome.

Many of the *sus* mutants of CAMPBELL cannot be used to analyze the left arm in a quantitative manner. This is due to the fact that CAMPBELL's mutants give small burst sizes under permissive conditions, suggesting that the mutant phenotype is not fully suppressed in the permissive host. To obtain reproducible recombination values in order to establish the size and spatial arrangement of genes in the left arm of the λ genome, the burst size of *sus* mutants under permissive growth conditions should approach that of lambda wild type. Therefore, new conditions for performing crosses have been devised and new *sus* mutants have been isolated which appear to be fully suppressed in the permissive host. The complementation between genes in the left arm has been studied by measuring the total phage yield of non-permissive cells which have been infected with two different *sus* mutants. Evidence is presented that the left arm of the lambda

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chromosome contains at least 18 cistrons and that three groups of genes in this portion of the lambda genome are co-transcribed.

MATERIALS AND METHODS

Strains used: The bacterial strains used were: C600 (APPLEYARD 1954), 594 (WEIGLE 1966) and W3350 gal 1⁻ gal 2⁻ pm⁻ (CAMPBELL and BALBINDER 1958). C600 is permissive and 594 is restrictive for λ *sus* mutants.

All of the λ mutants isolated in this laboratory were derived from the lambda PaPa strain of KAISER (1957). A series of λ *sus* mutants obtained from DR. A. CAMPBELL was used to define genes A-J in preliminary complementation tests. Temperature-sensitive (*ts*) mutants of lambda were obtained from J. WEIGLE and A. HARRIS. In addition, *ts* mutants defective in cistrons V, H, L and I were isolated in this laboratory among *sus*+ revertants of *sus* mutants defective in those genes. All of the *ts* mutants could grow on C600 or 594 at 37°C but not at 42°C.

Media: Nutrient agar plates for phage and bacterial assays contained: Bacto-agar (Difco) 10 gm; BBL trypticase (Baltimore Biological Laboratories) 10 gm; NaCl 5 gm in one liter of distilled H_2O. BBL soft agar is of the same composition except that 6.5 gm of Bacto-agar is used per liter.

All bacteria were grown in K medium, which is M9 buffer (ADAMS 1959) containing 1.5% charcoal-filtered casamino acids (Difco), 0.005 M $MgSO_4$, and 0.05% NaCl. For adsorption of λ particles, bacteria were grown in K medium containing 0.2% maltose (KM); otherwise, K medium containing 0.2% glucose (KG) was used. For growth of C600, thiamine hydrochloride was added to a final concentration of 10 μg/ml.

Dilutions were done in TMG buffer which contains 0.01 M tris HCl, pH 7.4, 0.01 M $MgSO_4$, and 0.01% gelatin.

Plating bacteria: Phage were assayed with bacteria grown to stationary phase in KM, chilled, centrifuged, and resuspended in 2/3 volume of 0.01 M $MgSO_4$.

Crosses and liquid culture complementation: A saturated culture of C600 for crosses or 594 for liquid culture complementation tests was diluted 2000-fold into KM at 37°C and grown with aeration to a density of 5×10^7–1×10^8 cells per ml. The bacteria were chilled, centrifuged, and resuspended in cold TMG at 4×10^8/ml. Log phase bacteria prepared in this manner give much higher phage yields than do stationary phase bacteria. A mixture of the two parental phages at 4×10^9/ml is added to an equal volume of bacteria to give a concentration of bacteria at 2×10^8/ml and a multiplicity of infection of 10. Adsorption is carried out at 37°C for 15 minutes. Under these conditions, phage adsorption is better than 95% when bacteria have been grown in maltose. The complexes are then diluted 4×10^4 into KG at 37°C and incubated for 90 minutes. The use of antiserum to inactivate unadsorbed phage and chloroform to promote lysis did not alter either the background or the final yield and was omitted in most experiments.

Mutagenesis and isolation of sus *mutants:* Two different mutagenic treatments were used. In one, a stock of lambda wild type (λ++) was treated with hydroxylamine following the procedure of FREESE, BAUTZ-FREESE and BAUTZ) (1961). In the other treatment, 594 carrying a temperature-inducible lambda prophage (λCItl, LIEB 1964) was grown to 2×10^8/ml in KG at 37°C. The prophage was induced by placing the cells at 43.5°C for 20 minutes. The induced cells were returned to 37°C and N-methyl-N'-nitro-N-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wisconsin) was added to a final concentration of 5 μg/ml. The culture was aerated until lysis and chloroformed.

sus mutants were selected by plating the treated phage stocks on C600 and overlaying with soft agar containing an equal amount of 594. Since the *sus* mutants grow only on C600, they make uniformly turbid plaques which can be easily distinguished from the plaques made by *sus*+ phage.

Isolation of λdg and λdg spot tests: A series of W3350 (λdg) lysogens was made from a lysate of UV-induced C600 (λ++) following the general procedures described by CAMPBELL (1958). To order mutant sites by deletion mapping with λdg's (CAMPBELL 1959) an agar plate was seeded

with 0.15 ml of W3350 (λdg) and 0.15 ml of 594, both of which had been grown to saturation in KM. The plates were spotted with a drop containing about 2×10^6 particles of the unknown mutant and allowed to dry, after which each plate was UV-irradiated with the dose used for optimal induction of liquid cultures. Each mutant was also spotted on 594 alone as a control. The plates are incubated overnight at 37°C.

The UV-irradiation serves two purposes in this test: the λdg prophage is induced and immunity to superinfection is destroyed; and secondly, recombination between the superinfecting *sus* mutants and λdg is increased several fold. Since *sus* mutants cannot grow on W3350 or 594, a positive test (an area of lysis on the plate) can only result if the *sus* mutation lies outside the deletion so that *sus+* recombinants can be formed.

Complementation spot tests: Spot tests were done by seeding a plate with 594 and then spotting the plate with a drop containing approximately 2×10^6 particles of each of two different *sus* mutants. The plates are incubated overnight at 37°C. A positive test—an area of lysis—indicates that the two *sus* mutants are defective in different genes. Recombination between *sus* mutants defective in the same gene to produce *sus+* phage will not give a positive test under these conditions.

RESULTS

Isolation and classification of new sus *mutants: sus* mutants were selected after growing the mutagen-treated stocks for several cycles on the permissive strain C600 to eliminate the possibility of mutational heterozygotes. Uniformly turbid plaques were transferred from the double layer plates with a sterile pin onto plates seeded with 594 and C600, respectively. Phage that grew on C600 but did not grow on 594 were purified from the C600 plate by a single plaque isolation. Approximately 0.3% of the phage that survived either mutagenic treatment were *sus* mutants, however no precautions were taken to insure that the mutants arose independently. The level of spontaneous *sus* mutants which would have been detected with this technique was found to be less than 0.01% in untreated control samples. A total of 310 *sus* mutants was isolated and stocks were prepared by the confluent lysis method (ADAMS 1959) for further studies.

A three-step screening procedure was employed to determine the complementation class and approximate location of each *sus* mutant in the left arm. The following procedure applies only to *sus* mutants in the left arm of the lambda chromosome.

(1) Each *sus* mutant was first spot tested against a series of λdg prophages whose deletion end-points had been previously established by tests with the reference *sus* mutants defective in genes A through J provided by DR. A. CAMPBELL. This allowed each mutant to be assigned to a group of two or three adjacent genes within the left arm.

(2) Complementation spot tests were done using six reference *sus* mutants defective in genes spanning the region indicated by the λdg test. This test was almost always adequate for making a definite cistron assignment for each new *sus* mutant.

(3) Many of the *sus* mutants were further tested by liquid culture complementation. In this test, non-permissive bacteria were infected with equal multiplicities of a reference *sus* mutant and an unknown *sus* mutant, and the total phage yield produced during one lytic cycle was measured. Two mutants defective

in the same gene typically produce fewer than 1/10 of a phage per infected cell. Two *sus* mutants which complement each other yield 100–250 phage per cell, indicating that the mutants are defective in different functions and therefore different genes. Some pairs of *sus* mutants, however, produce intermediate yields of 10–30 phage per cell. These combinations will be discussed in detail in the section on polarity.

Complementation classes in the left arm: A summary of the new *sus* mutants obtained in this study is presented in Table 1. No new *sus* mutants were obtained in cistron D or in cistron T, a new gene between G and H (MOUNT, *et al.* 1968). *sus* mutants defective in four additional new genes were isolated; these genes

TABLE 1

New sus *mutants in the left arm of the lambda chromosome*

Cistron	Number of isolates	Number of sites	Order of mutant sites*
A	4	4	—11†—914—420—(701—854)—
W	6	3	—403,748,805,822—859—812—
B	2	2	—915—427—
C	5	5	—737—884—434—407—818—
D	none	. .	—15†—123†—
E	2	2	—815—723—
F	17	7	—785,826,865—471,478—730,893—421,889— —804,910—423,431,739,762,876—784—
Z	11	5	—718,735,740—405,712,834,916—744,758— —453—797—
U	6	5	—733,897—869—413—(824—858)—
V	11	4	—(438,466,475,817,852,877—750,760—736— —769,873)—
G	15	9	—447—703,917,919—764,799—759,872— —832—825—436,880—874,898—901—
T	none	. .	—*ts*40‡—
H	10	5	—866,885—706—435—717,745—437,457,463,848—
M	3	3	—888—481—793—
L	16	7	—756,853,908—771—780,860—441,708, 725,836—439—778—749,814,841,850—
K	13	10	—429—768,819—770,847—763—424—702— —755—(704—761,833)—892—
I	2	2	—811—838—
J	7§	5	—442—449—486—418,428,433—426—

* The cistrons are listed in the order in which they occur on the genetic map; each mutant site is separated by dashes, and sites enclosed in parentheses have not been ordered with respect to one another. Mutants numbered between 401 and 488 were induced with hydroxylamine; those numbered from 700–922 were induced with nitrosoguanidine. Two mutations were considered to be at the same site if no recombination could be detected between them after each parent had been UV-irradiated with a dose of two phage lethal hits, which increases recombination frequencies approximately 20-fold.

† These mutants were obtained from A. CAMPBELL and were used to construct a map of the left arm. They are shown in their correct order, but have not been included in the columns headed "Number of isolates" or "Number of sites."

‡ Cistron T has been defined by temperature-sensitive mutants (MOUNT, *et al.* 1968), and *ts*40 from A. HARRIS was used to establish the map position of the T cistron.

§ 30 mutants were isolated in the J cistron, but only 7 of these have been analyzed in detail.

are designated by the letters W, Z, U and V in accordance with the nomenclature proposed by CAMPBELL (1961). Gene U has been identified independently by MOUNT, *et al* (1968) using *ts* mutants.

The distribution of mutant isolates among the 78 sites shown in Table 1 closely approximates a Poisson distribution with a mean of 0.75, which indicates that more than one-half of the sites that could be defined by this type of *sus* mutation have already been found. Moreover, the mean number of sites per gene is 5 and none of the 16 genes defined by this class of *sus* mutants has fewer than two mutational sites. This suggests that few new genes would be found by isolating more *sus* mutants of this type.

In vitro *phenotypes of genes in the left arm:* The *in vitro* complementation test devised by WEIGLE (1966) can be used to determine one aspect of the defective phenotypes of *sus* mutations in the left arm of the lambda chromosome. WEIGLE has shown that *sus* mutants defective in genes A, B, D and E prevent the assembly of functional phage heads; *sus* mutants defective in genes G, H, M, L, K, I and J prevent the synthesis of functional phage tails. In the terminology of WEIGLE, genes A, B, D and E are "tail donors" and genes G, H, M, L, K, I and J are "head donors" because defective lysates of mutants in these genes produce normal tails and normal heads, respectively. At the time, cistron F could not be classified in this manner because the available mutants defective in the F cistron were very leaky.

An *in vitro* complementation analysis has been made of *sus* mutants defective in cistrons W, C, F, Z, U and V to complete the classification of the phage structural genes in this respect. Although the T cistron was not studied, it appears to control a step in phage tail synthesis (KEMP, HOWATSON and SIMINOVITCH 1968). The data summarized in Table 2 show that W, C and F mutants only make phage tails under restrictive conditions, and must, therefore, control the production of functional phage heads. Cistrons Z, U and V are seen to be head donor genes, indicating that these genes are involved in the formation of complete

TABLE 2

In vitro *complementation patterns of previously untested cistrons*

Mutant lysate	Phage made after mixing a mutant lysate with: Head donor (L_{63})	Tail donor (A_{11})	Self (Background)
W_{403}	250	<.001	<.001
C_{434}	410	<.001	<.001
F_{423}	200	<.01	<.01
Z_{405}	<.01	350	<.01
U_{413}	<.001	560	<.001
V_{438}	<.001	310	<.001

Defective lysates were made by growing 594 carrying a temperature-inducible *sus* prophage to 2×10^8 in KG at 37°C. The cells were induced at 43.5°C for 20 min and returned to 37°C until lysis occurred. *In vitro* complementation was measured by mixing equal volumes of a mutant lysate with a head or tail donor lysate (see WEIGLE 1966). The reaction was stopped after 3 hours incubation at room temperature and the total phage were assayed. The data give the number of phage made $\times 10^{-7}$.

phage tails. Thus all of the genes in the left arm control the assembly and structure of the mature lambda particle, which further strengthens the notion (see DOVE 1966) that the lambda chromosome is organized into groups of genes whose functions are related.

A genetic map of the left arm: To insure that the crossing procedure described in the METHODS section was indeed valid for genetic analyses of the new *sus* mutants, some of the factors which can influence the measurement of recombination frequencies were checked. The reversion frequencies and leakiness of the *sus* mutants used in a cross limit the accuracy with which one can measure sus^+ recombinants among the many *sus* progeny of that cross. The reversion frequencies of the new *sus* mutants range between 10^{-5} and 10^{-8}, and with the exception of several sites in the F cistron, none of the mutants are leaky—that is, the burst size of these *sus* mutants in single infection of a non-permissive host is very much less than one. Thus all of the new *sus* mutants are suitable for genetic mapping.

The generation and formation of wild-type recombinants in a cross does not seem to be favored under the crossing conditions used here. To show this, a series of crosses was performed between *sus* and sus^+ phage and the ratio of *sus* to sus^+ phage among the progeny was compared to that of the parental input. The allele ratio among the progeny was always the same as that of the parental input over a range of allele ratios from 1:10 to 10:1. Thus lambda wild type does not have a selective advantage in mixed infection with a *sus* mutant under the conditions employed.

The left arm of the lambda genome was mapped by crossing several mutants defective in a gene by mutants defective in the two nearest genes on either side of that gene. The position of mutant sites within each gene was established by three-factor crosses in which the CI gene (KAISER 1957) was employed as an unselected outside marker by using the wild type (CI^+) and temperature-inducible (CIt1) alleles of the CI gene. At 42°C phage carrying the CIt1 mutation form clear plaques but phage with the CI^+ allele make turbid plaques. The segregation of the outside marker among the sus^+ recombinants was followed by plating the progeny from a cross on 594 at 42°C and scoring clear and turbid plaque-formers. This method was reliable for ordering mutant sites which are separated by at least 0.1% recombination. Markers closer than this could not be reliably ordered with respect to the outside marker because of high negative interference (AMATI and MESELSON 1965).

In order to evaluate the effects of negative interference on the additivity of recombination distances, and to determine whether a mapping function (STAHL, EDGAR and STEINBERG 1964) could be constructed, the results of a representative sample of 2-factor crosses were analyzed as shown in Figure 1. The data indicate that recombination values are very nearly additive from 0.1% to about 3%. Apparently, negative interference does not appreciably affect the additivity of recombination distances derived from 2-factor crosses. Since a mapping function was unnecessary, the map of the left arm shown in Figure 2 was constructed by

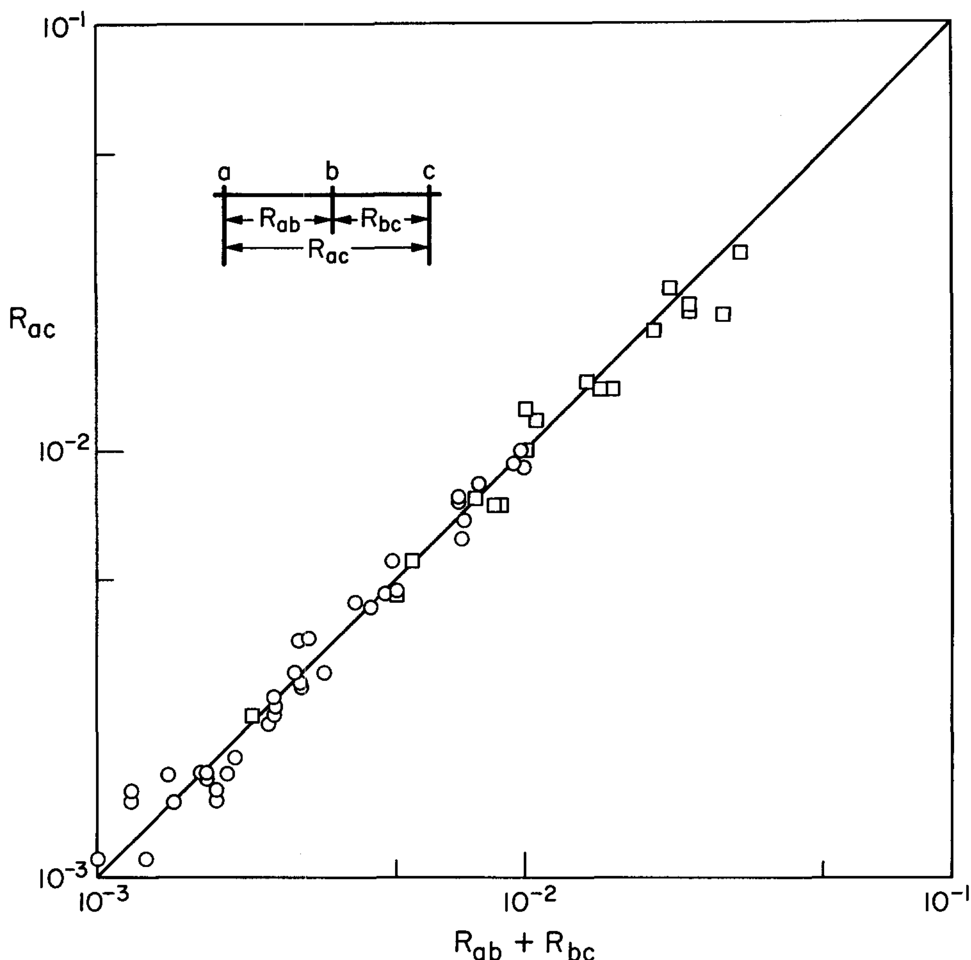

FIGURE 1.—The additivity of recombination distances in the left arm of the lambda chromosome. The frequency of recombination (R) is defined as 2 × plaques on 594/ plaques on C600 for the progeny of a cross. The frequency of recombination between sites *a* and *c* (R_{ac}) has been plotted on log-log paper as a function of the sum of the primary intervals R_{ab} and R_{bc}. The data are taken from a sample of two-factor crosses between *sus* mutants in the left arm. Burst sizes in these crosses usually ranged from 150–300 phage per cell, and no cross was accepted if the burst size was below 100 phage per cell. ○ = intragenic crosses; □ = intergenic crosses.

converting recombination frequencies directly into relative distances on the genetic map.

Polar effects between genes in the left arm: The left arm of the lambda chromosome is divided into two non-overlapping gene clusters: genes A-F which synthesize the mature phage head; and genes Z-J which synthesize the functional phage tail. We can further characterize this portion of the λ genome at the level of transcription by studying polar effects among the genes of this region. The evi-

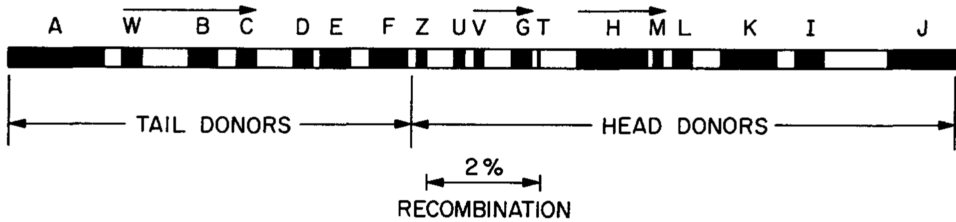

FIGURE 2.—A genetic map of the left arm of the lambda chromosome. The observed recombination frequencies between *sus* mutants in the left arm were converted to relative distances on the genetic map. The shaded areas represent the minimum estimate of the size of a gene based on recombination between the most distant *sus* mutants defective in that gene. Genes A-F are tail donors and genes Z-J are head donors as determined by *in vitro* complementation patterns (WEIGLE 1966). The extent and orientation of polarity in this region of the genome is indicated by the arrows above some of the cistrons.

dence, from work on bacterial operons, strongly suggests that polarity—the reduction of one gene's activity by a nonsense mutation in a neighboring gene—is due to the fact that some genes are transcribed and translated together as a unit (IMAMOTO and YANOFSKY 1967a, b). The inability of nonsense codons to specify an amino acid under non-permissive conditions somehow prevents the expression of any genes beyond the nonsense mutation when these genes are organized as a unit of transcription. The basis of polarity may be the postulated coupling of transcription and translation (STENT 1966) and it is clear that in bacterial operons, the orientation of polar effects is the same as the direction of transcription and translation.

STAHL *et al.* (1966) have demonstrated with phage T4 that the phage yield from non-permissive cells which have been mixedly infected with *sus* mutants defective in different genes can be used as a measure of polarity. If the two mutants are not polar, the phage yield should be identical whether the functioning wild-type alleles of these genes are on the same (*cis*) or on different (*trans*) chromosomes. A low yield in the *trans* configuration compared to the *cis* control can be indicative of polarity. *Cis-trans* tests such as this have been done with each pair of adjacent genes in the left arm and the results (Table 3) indicate that mutations in cistrons W-B, B-C, V-G and H-M may be polar since the complementation between mutants defective in these genes is reduced when the mutations are in the *trans* configuration.

The polarity of cistrons W-B, B-C, V-G and H-M has been confirmed by demonstrating that poor complementation between mutants defective in these genes is due to the presence of a *sus* (nonsense) mutation in only one of the genes of each pair. One way to show this is to replace the *sus* mutants with *ts* mutants and repeat the *trans* complementation tests to see whether the *cis-trans* position effect still occurs (see STAHL *et al.* 1966). The results of this test for gene pairs B-C, V-G and H-M are shown in Table 4. In each case one combination of *ts* × *sus* no longer exhibits poor complementation, whereas the opposite combination still complements poorly. The example, U-V, has been included to show that a gene

TABLE 3

Cis-trans *tests of adjacent genes in the left arm*

Cistrons tested	*Cis* test $\left(\frac{+\ \ +}{sus\ \ sus}\right)$ Percent of λ^{++} yield	*Trans* test $\left(\frac{+\ \ sus}{sus\ \ +}\right)$ Percent of λ^{++} yield	Percent of *cis* yield
A × W	76(2)	40(5)	53
W × B	53(4)	10(6)	*19*
B × C	45(4)	12(4)	*26*
C × D	.....	40(4)	..
D × E	.....	40(4)	..
E × F	76(2)	50(2)	66
F × Z	80(1)	50(2)	62
Z × V	100(2)	95(2)	95
U × V	62(1)	73(1)	117
V × G	82(1)	13(5)	*16*
H × M	54(4)	7(7)	*13*
M × L	85(2)	61(5)	72
L × K	64(1)	60(1)	94
K × I	50(1)	50(1)	100
I × J	52(2)	40(4)	77

594 was infected with 5 particles of each parent and the total phage yield was measured after 90 min incubation at 37°C. Under these conditions the yield of a λ++ control ranges from 150–250 phage per cell. The figures in parentheses refer to the number of times each test was performed. The data are averages of all the tests, but the values usually did not vary more than 15% for tests done on different days using different mutants in a gene.

The double mutants were constructed by crossing two *sus* mutants and spot testing the progeny against each parent. Those progeny which did not complement either parent were then crossed by each parent to verify the genetic constitution of the double mutant.

TABLE 4

A directional test to confirm the polarity of B-C, V-G and H-M

Test		Gene 1–Gene 2 B-C	V-G	H-M	U-V (Control)
$\frac{+\ \ +}{sus1\ \ sus2}$	(*cis* control)	100	80	50	65
$\frac{ts1\ \ +}{+\ \ ts2}$	(*ts trans* control)	70	45	50	55
$\frac{sus1\ \ +}{+\ \ sus2}$	(*sus trans* control)	25	10	10	50
$\frac{ts1\ \ +}{+\ \ sus2}$	(Experimental)	65	50	50	60
$\frac{+\ \ ts2}{sus1\ \ +}$	(Experimental)	25	10	10	50

594 was infected with 5 particles of each parent and the total phage yield was measured after 60 min incubation at 41.5°C. The data are expressed as per cent of a λ++ control yield, which is about 50 phage per cell at this temperature. Mixed infections of mutant and wild type were also done as controls. These always gave at least 60% of the wild-type yield for the mutants described in this table.

pair which exhibits no *cis-trans* differences in *sus* × *sus* tests also complements well in both *ts* × *sus* configurations. These data show that a *sus* mutant defective in cistron B, for example, prevents the full expression of cistron C which lies adjacent to B on the genetic map. However, a *sus* mutant defective in C does not affect the activity of cistron B. Thus the results show that poor complementation between *sus* mutants defective in genes B-C, V-G or H-M is caused by polarity. Each effect is unidirectional, which indicates that the direction of m-RNA synthesis most likely proceeds from B to C, from V to G, and from H to M.

The test described above could not be used with gene pair W-B because no *ts* mutant was available in the W cistron. A different test has been used to show that genes W and B are polarized. In this experiment, non-permissive bacteria were infected with *sus* mutants defective in cistrons W and B, and although the total multiplicity of infection was held constant, in separate tests different ratios of *sus* W to *sus* B phage were used to infect the cells. The burst size was then measured as a function of the number of *sus* W phage in the infected cells. STAHL *et al.* (1966) have shown that this test can be used to determine the direction of a polar effect. If, for example, *sus* mutants in W affected the expression of B, we would expect the burst size to increase with an increase in the number of *sus* W genomes in the infected cells, since more opportunities would exist for transcription and translation of the B cistron to occur. If W and B are not polarized, we should find that the burst size reaches a maximum when there is an equal number of *sus* W and *sus* B genomes in the infected cells. The results shown in Figure 3 demonstrate that a *sus* mutant defective in cistron W does decrease the expression of cistron B, since the burst size is directly dependent on the number of *sus* W mutants in the infected cell. The curves shown in Figure 4 demonstrate, as predicted, that non-polar genes complement most efficiently when the two parental inputs are equal in number.

The controls of $\lambda sus \times \lambda^{++}$ were also done to insure that input gene dosage or stoichiometric effects do not account for the differences in the results of Figures 3 and 4. The input ratio of *sus* to sus^{+} phage was varied from 1:8 to 8:1 and the dosage dependence of each gene product was estimated from the dependence of burst size on the number of sus^{+} genomes in the infected cells. None of the mutants tested exhibited gene dosage effects sufficient to account for the differences in the results presented in Figures 3 and 4.

Since W-B and B-C are polar, we would also expect W and C to be polar, and in the direction W to C. The results of Figure 3 confirm this notion, since the burst size in mixed infection by *sus* W and *sus* C phage is directly dependent on the number of *sus* W genomes in the infected cells. Thus, cistrons W-B-C constitute a polar triplet and are probably transcribed into messenger RNA as a unit.

Polarity studies on bacterial operons have shown that the position of a nonsense mutation within a gene determines the degree of polarity of that mutation. There exists a gradient of polarity throughout each gene of an operon such that mutations near the beginning of a gene cause much greater polar effects than do nonsense mutations near the end of that gene (NEWTON *et al.* 1965). Two *sus* mutants—*sus*403 and *sus*812—defective in gene W have been examined in com-

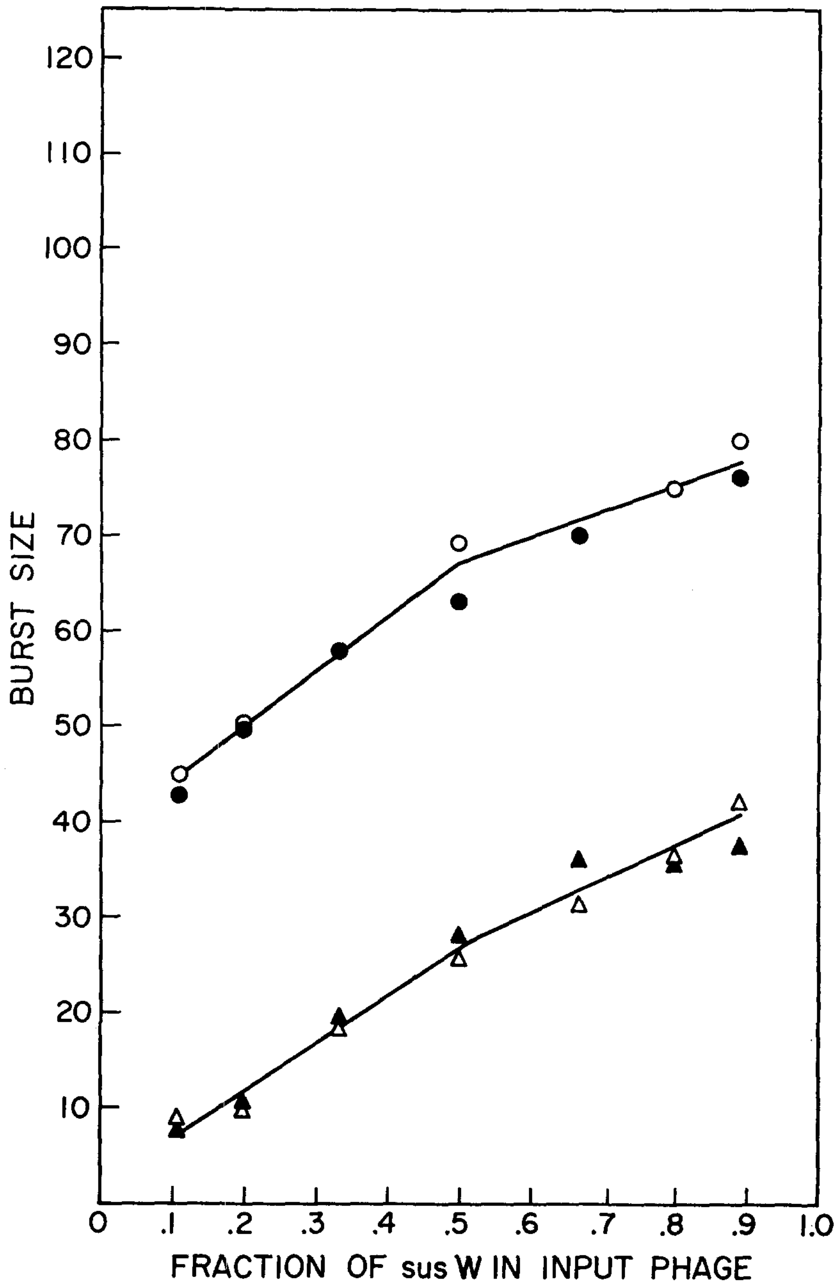

FIGURE 3.—The dependence of burst size on input ratio for polar gene pairs W-B and W-C. 594 was infected with two *sus* mutants at a total multiplicity of infection = 10. The input ratio of the two *sus* phages was varied from 1:8 to 8:1. After adsorption and anti-λ serum treatment to inactivate unadsorbed phage, the infected cells were plated on C600 before lysis to determine the number of cells yielding progeny phage. The total phage yield from these cells was measured after 90 min incubation at 37°C in KG medium. The burst size is defined as the ratio of total phage to cells which produced phage. △, ▲ $sus_{W_{403}} \times sus_{B_{427}}$ ○, ● $sus_{W_{403}} \times sus_{C_{434}}$

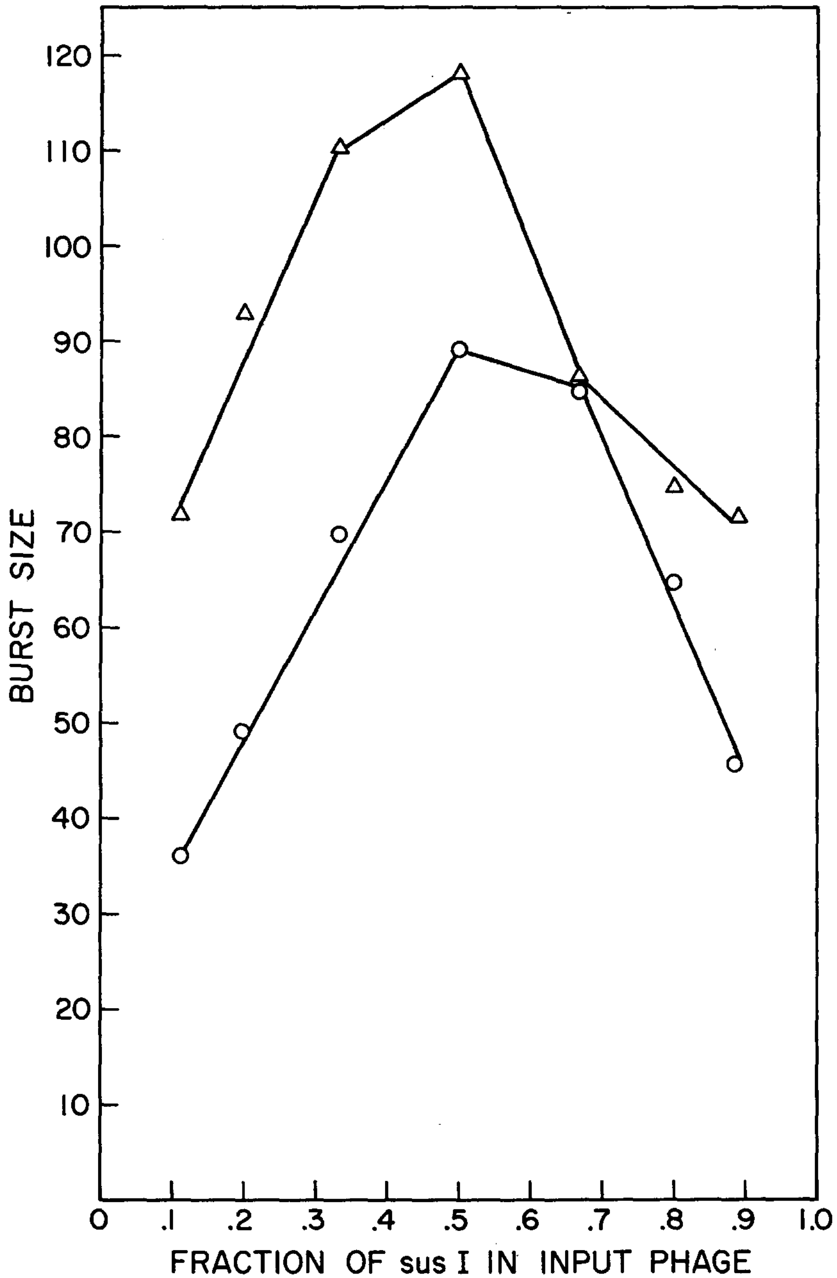

FIGURE 4.—The dependence of burst size on input ratio for non-polar gene pairs I-K and I-J. The procedure used was the same as that of Figure 3. ○ = $sus_{\mathrm{I}_{811}} \times sus_{\mathrm{J}_{418}}$ △ = $sus_{\mathrm{I}_{811}} \times sus_{\mathrm{K}_{424}}$

plementation tests with a *sus* mutant defective in cistron B to show that a gradient of polarity exists in cistron W. Five experiments were done with each *sus* mutant. *sus*403, which lies 0.25 recombination units to the left of *sus*812 on the genetic map, produces 7.8% (S.D. = 2.2%) of the wild-type yield in mixed infection with a *sus* B mutant. *sus*812 produces 13.1% (S.D. = 1.2%) of the wild-type yield in mixed infection with the same *sus* B mutant, indicating that a small gradient of polarity seems to exist within the W cistron. Since the burst size is a poor measure of any particular gene product, owing to the numerous interactions between phage precursors which give rise to an infective particle, this effect may be much more striking at the gene product level. NAKATA and STAHL (1967) have observed polarity gradients of a similar magnitude in phage T4.

DISCUSSION

It is quite probable that the 18 genes which have been defined by complementation between mutants in the left arm of the lambda chromosome account for nearly all of the genetic material in this region of the genome. If we assume that an average gene in this region contains 1,000 nucleotide pairs, and that this region is about ⅓ of the lambda chromosome or 20,000 nucleotide pairs long, we would expect about 20 genes in the left arm. Furthermore, there are no large gaps, as measured by recombination, between any of the known genes in the left arm of the lambda chromosome. Finally, the number of mutant sites per gene suggests that no more genes will be discovered with the type of *sus* mutants described in this paper.

Polar effects between *sus* mutants defective in adjacent genes such as those described here for phage λ have also been demonstrated in phage T4 by STAHL *et al.* (1966). In both phages a quantitative analysis is made difficult by gene dosage or precursor concentration effects, but it appears as though the basis for the *cis-trans* effects that have been observed in λ and T4 is in fact polarity of the same sort as that found in bacterial operons. Polar genes in both T4 (NAKATA and STAHL 1967) and lambda exhibit gradients of polarity. Moreover, in the lambda system the direction of transcription has recently been determined by examining the strand-specificity of messenger RNA made at early and late times during the vegetative cycle (TAYLOR, HRADECNA and SZYBALSKI 1967; COHEN and HURWITZ 1967). The left arm, which is only transcribed late in infection, is read in the direction from cistron A to cistron J. This is also the orientation of polar effects among three groups of genes in this portion of the lambda chromosome, and is consistent with the notion that the direction of transcription and the direction of polarity are the same.

In phage lambda the genes of the left arm are arranged into two non-overlapping clusters: one group of 7 genes concerned with DNA maturation and head formation; and another group of 11 genes which control phage tail assembly. The relationship, if any, between the clustering of functionally similar genes and the control of development during the final stages of the lambda growth cycle is not yet understood. It may be that the structural genes of the left arm are

expressed in a temporal sequence that parallels the order of those genes on the chromosome. The steps in the process of assembling mature lambda particles may also take place in the same sequence as the genes which control each step occur on the lambda chromosome.

Although previous polarity studies with phage T4 (STAHL *et al.* 1966) have failed to detect polar effects that involved more than two adjacent genes, the discovery in phage λ of polar genes W-B-C demonstrates that polarity need not be restricted to only two adjacent genes. The importance of joint transcription in phage morphogenesis is not known, but one possibility is that polarity provides a mechanism which insures that several critical gene products will be made at the same time, and in the correct relative amounts, during phage assembly. Phage lambda provides an excellent system with which to test this hypothesis.

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SUMMARY

The left arm of the chromosome of phage lambda was studied by using newly-isolated *sus* nonsense mutants. Four new genes were found which brings to 18 the number of known genes in this portion of the lambda chromosome. Recombination distances from two-factor crosses were found to be nearly additive in this region which allowed the construction of a genetic map of the left arm without the use of a mapping function. Physiological studies of these genes reveal two functional classes of genes in the left arm. Seven genes control phage head formation and another eleven genes are responsible for phage tail morphogenesis. It is clear from the mapping studies that the head genes are arranged into one cluster and the tail genes into another cluster on the chromosome. *Cis-trans* tests showed that three groups of genes in the left arm are polar, two polar groups contain two genes each and another polar group is composed of three genes. The orientation of the polarity effects is in all cases the same as the direction of transcription.

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