GENETIC FINE STRUCTURE OF THE C REGION AND THE LINKAGE MAP OF PHAGE P22[1]

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ON infection with "temperate" phage, some bacteria may lyse, but some survive and give rise to lysogenic progeny. Lysogenic bacteria possess the ability to produce the infecting phage but are immune to subsequent infection by the phage. Genetic control of the lysogenic condition lies in the prophage which becomes part of the hereditary material of the bacterium (LEDERBERG and LEDERBERG 1953; LENNOX 1955; JACOB 1955). Temperate phages usually produce plaques with turbid centers owing to the growth of lysogenic bacteria. "Virulent" phages produce plaques with clear centers as they usually lyse all infected cells with concomitant liberation of progeny phages.

The wild type of phage P22 of *Salmonella typhimurium* (hereafter designated c^+) is temperate and produces turbid plaques (ZINDER and LEDERBERG 1952). Clear-plaque forming mutants, affected in the ability to become prophage, have been isolated. All such mutants fall into one of three phenotypic groups; c_1, c_2 and c_3 (LEVINE 1957). The mutants of class c_3 are still temperate, but the frequencies of lysogenization with these are lower than with c^+. The clear mutants c_1 and c_2 behave like virulent phages. However, in mixed infection with mutants of the c_2 type, phages of the c_1 type lysogenize as well as the c^+ phage. This interaction between virulent phages in mixed infection resulting in lysogeny has been called co-operation (KAISER 1957). Preliminary genetic studies placed the c mutations in a localized region of the phage P22 linkage map. The present report describes a detailed fine mapping of this region leading to its division into three complex genetic loci corresponding to the three phenotypic groups of mutants.

MATERIALS AND METHODS

Bacteriophage strains: The c^+ type of the *Salmonella typhimurium* phage P22, a large number of independently arising spontaneous c mutants, and some plaque-morphology mutants were used.

A complete description of the characteristics of the three classes of c types and a spot test for their rapid identification has been given (LEVINE 1957). No ex-

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ceptions to this classification have been detected among the more than 150 spontaneous *c* mutants tested. The plaque-morphology mutants, as scored on indicator agar (see below) have the following characteristics:

+:	wild type; dark green ring
m_1:	pale green ring with somewhat irregular edge
m_3:	dark green ring with golden brown halo
m_4:	brown-green ring with irregular edge
h_{21}:	narrow pale green ring with smooth edge; called *y* by ZINDER
g:	broad dark green ring with smooth edge
m_3h_{21}:	narrow pale green ring with golden brown halo
m_3g:	broad dark green ring with golden brown halo
m_1m_3:	pale green ring with golden brown halo
gh_{21}:	narrow dark green ring with smooth edge
gm_4:	darker green than m_4; somewhat similar to wild type but with a brownish cast and an irregular edge.

h_{21} with m_1 and m_4 cannot be distinguished by inspection.

Bacterial strains: Salmonella typhimurium strain LT2 and its mutants were used as host bacteria in these experiments.

Media: The following media were employed: L broth, indicator agar (BRESCH 1953), soft agar for top layers, EMB galactose agar and buffered saline. The methods of preparation of these media are as previously described (LEVINE 1957).

Experimental procedures

Galactose positive bacteria of strain LT2 are grown to a concentration of about 10^8 cells/ml in L broth. The cells are washed twice in buffered saline, resuspended to one half the original volume and aerated for 45 minutes at 37°. The cell concentration is then readjusted to 10^8 cells/ml with buffered saline. These bacteria are infected with equal multiplicities, ten particles, of each of the two parent phage stocks. Adsorption is at least 95 percent completed in five minutes. A 1:10 dilution is made in L broth containing antiphage serum in concentration sufficient to inactivate 99 percent of the unadsorbed phage in five minutes. Further dilutions are made and 0.05 ml samples are plated on EMB galactose agar with a heavy inoculum of a Gal^- phage sensitive mutant. Plating on EMB galactose agar permits the detection of infective centers and surviving bacteria which may or may not be lysogenic (LEVINE 1957). After 90 minutes, when the rise period is completed, chloroform is added to the dilute lysate to kill any surviving bacteria. Diluted samples are plated on indicator agar for examination of the phage liberated by lysis. Mixtures of parental phages are irradiated in buffered saline before infection with ultraviolet light in some of the crosses to be reported below. The ultraviolet source used was a 15 watt G.E. germicidal lamp at a distance of 50 cm for exposures of 105 seconds. Approximately five percent of the exposed particles survive this dose.

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RESULTS

Crosses were undertaken between a large number of c mutants to study the genetic structure of the c region. The expected recombinants between two non-identical c mutants are the double mutant and its reciprocal, the turbid c^+. The double mutants are not easily distinguished among the progeny and are therefore not of much use in such studies. On the other hand, the turbid plaques of the c^+ recombinants are easily detected and the frequency of these among the total progeny of a mixed infection can be determined with accuracy. In addition, this determination is not complicated by back mutation as no case of reversion from c to c^+ is known in this material. Some 300,000 progeny from control infections with all of the individual mutants used in these experiments have been carefully screened without observing any c^+ phages. Many of these were exposed to ultraviolet light before infection and still no reverse mutations were found. Many tens of thousands more have been examined less carefully with the same result. It seems quite clear that these c^+phages arise by recombination as they are found only among the yields of mixed infection with independently isolated c mutants. The frequencies of c^+ recombinants from these mixed infections are, then, taken as the measure of linkage between parental c mutants and these frequencies are doubled (on the assumption that the reciprocal double mutants are formed as frequently) in the data to be presented.

The c^+ recombination data (Table 1) from two factor crosses with 16 c mutants support the previous conclusion (LEVINE 1957) that the c mutants fall into a circumscribed region of the phage chromosome. The distribution of individual mutations within this region is, however, not random. Rather, there is a clustering of mutations of similar phenotype, dividing the region into three parts. Into one genetic group fall all the mutations previously classified as type c_1. These are closely linked with the region into which all the c_2 type mutations fall. These in turn are linked to the region in which the three c_3 mutations lie. Within each of these groups, the individual mutations map as specific sites which can be arranged in a linear order. The c region, then, is made up of three closely linked complex genetic loci, c_1–c_2–c_3, corresponding to the three phenotypic classes of c mutants. Each locus consists of multiple sites at which mutations may occur, affecting that particular phenotype, and these sites may recombine with one another. These facts are graphically illustrated by the linkage map of the c region in Figure 1.

To extend the fine mapping of the c region, use was made of the finding (JACOB and WOLLMAN 1955) that ultraviolet irradiation of parental phages before infection results in increased frequencies of phage recombination. The dose employed gives maximum recombination values in phage P22. The UV linkage map of the c region of phage P22 is shown in Figure 2. These results extend those given above and are in good agreement with them. Again there are no exceptions to the grouping of mutations according to phenotype into the three distinct loci. Also there is surprisingly good correspondence between the two maps in the order of the same mutational sites within these loci.

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TABLE 1

Genetic recombination in the lytic yield of two factor crosses between c *mutants*

Parents†	Total c^+ recombinants	Total progeny	Percent recombination (×2)
Intergroup crosses			
$c_1^{7} \times c_2^{5*}$	87	17,157	1.014
$c_1^{65} \times c_2^{5}$	37	15,377	0.481
$c_1^{49} \times c_2^{5*}$	214	50,747	0.843
$c_1^{65} \times c_2^{27}$	0	4,341	0.00
$c_1^{52} \times c_2^{27}$	24	11,125	0.432
$c_2^{5} \times c_3^{77*}$	103	25,381	0.812
$c_2^{5} \times c_3^{11*}$	41	8,099	1.01
$c_2^{5} \times c_3^{41*}$	85	7,499	2.27
$c_1^{65} \times c_3^{77}$	71	10,338	1.37
Intragroup crosses			
$c_1^{65} \times c_1^{60*}$	79	127,997	0.123
$c_1^{65} \times c_1^{49*}$	121	162,678	0.149
$c_1^{65} \times c_1^{7}$	96	126,627	0.152
$c_1^{65} \times c_1^{45*}$	111	111,873	0.198
$c_1^{65} \times c_1^{52*}$	106	96,632	0.219
$c_1^{60} \times c_1^{49}$	0	60,871	0.000
$c_1^{60} \times c_1^{7}$	0	97,807	0.000
$c_1^{60} \times c_1^{31*}$	17	94,216	0.0361
$c_1^{60} \times c_1^{52*}$	12	63,628	0.0377
$c_1^{60} \times c_1^{3*}$	37	110,861	0.0668
$c_1^{49} \times c_1^{52}$	38	122,656	0.0620
$c_1^{7} \times c_1^{52}$	15	122,970	0.0244
$c_1^{45} \times c_1^{52}$	1	113,596	0.0018
$c_1^{31} \times c_1^{3*}$	17	155,853	0.0218
$c_1^{52} \times c_1^{3}$	2	109,357	0.0037
$c_2^{5} \times c_2^{8*}$	153	116,474	0.263
$c_2^{5} \times c_2^{23*}$	143	103,756	0.276
$c_2^{5} \times c_2^{27*}$	463	185,659	0.499
$c_2^{8} \times c_2^{23*}$	15	125,848	0.0238
$c_2^{8} \times c_2^{27*}$	162	79,420	0.408
$c_2^{23} \times c_2^{27*}$	284	170,537	0.333
$c_3^{41} \times c_3^{77}$	0	4,300	0.00
$c_3^{41} \times c_3^{11}$	0	4,877	0.00
$c_3^{11} \times c_3^{77}$	0	5,892	0.00

† Parent phages were used at m.o.i. of ten each.
* Two other loci, m_3 and h_{21}, were followed in these crosses (see text).

Some c mutations behave anomalously in that they recombine with certain alleles to produce distinctive frequencies of c^+ progeny, but recombine with lower than expected frequencies or not at all with others. These comments are particularly pertinent to the c_3 locus. No c^+ recombinants are observed in the admittedly small samples of progeny from crosses between c_3^{11}, c_3^{77} and c_3^{41} (Table

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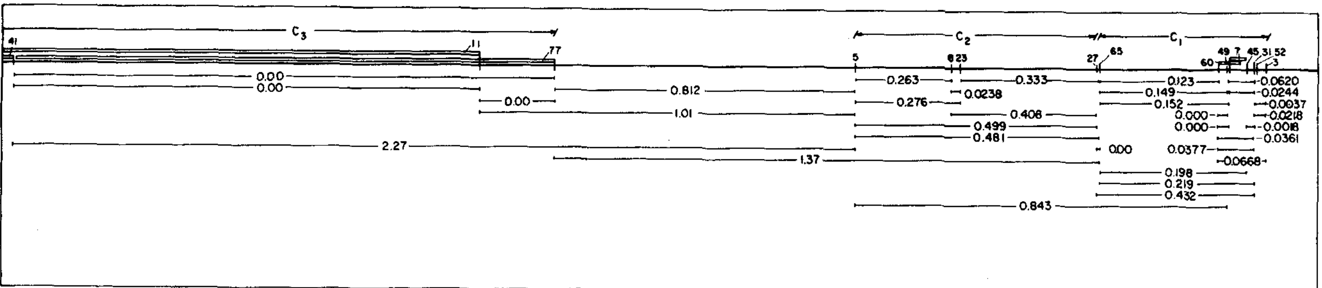

FIGURE 1.—Linkage map of *c* mutations. The order and distances are based on the uncorrected frequencies of recombination for the two factor crosses listed in Table 1. The open bars above the map indicate alleles which may be multisite mutations (see text).

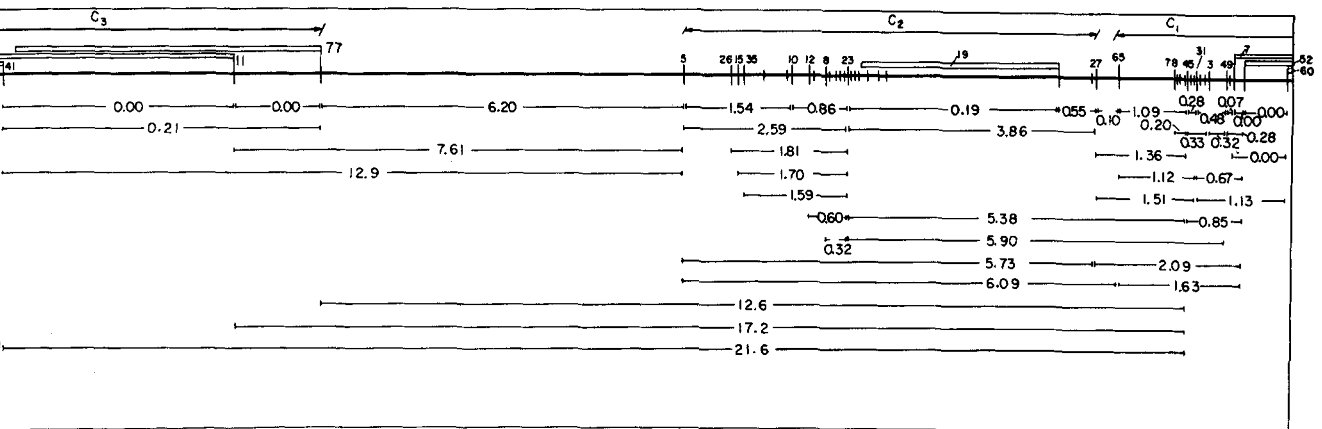

FIGURE 2.—The ultraviolet linkage map of *c* mutations. The map is based on recombination frequencies for two factor crosses in which mixtures of parental *c* phages are irradiated with ultraviolet light before infection. The position of the numbered alleles can be assigned with some assurance. The positions of unnumbered alleles (indicated on the map by the shorter perpendicular lines) are only approximate. The open bars above the map indicate alleles which may be multisite mutations (see text).

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1 and Figure 1). Following ultraviolet treatment, only 0.21 percent recombination is found in the $c_3^{77} \times c_3^{41}$ cross and no recombinants are observed in the other two crosses (Figure 2). These findings suggest that the c_3 locus is very small indeed. However, in crosses to mutant c_2^5 all three c_3 mutants give quite high frequencies of recombination: 0.812, 1.01 and 2.27 between c_2^5 and c_3^{77}, c_3^{11} and c_3^{41} respectively (Table 1 and Figure 1). These data suggest that the c_3 locus is rather long and that the individual c_3 mutations cover extensive and common segments. The possibility that the c_3 mutations (and a few others) are multisite mutations (DEMEREC and HARTMAN 1959) is indicated by the open bars above the linkage maps in Figures 1 and 2. These conflicting considerations make difficult the determination of the precise dimensions of the c_3 locus.

A number of four factor crosses have been carried out involving, in addition to the two c alleles, the m_3 and h_{21} plaque morphology markers. The order of the c mutations relative to one another and to the plaque morphology markers can be determined from the distribution of these markers and their wild-type alleles among the c^+ recombinant progeny. These crosses are designated by the asterisks in Table 1. Such analyses confirm the positions of the c mutations (as shown in Figure 1) wherever applicable and also establish the order m_3–c_3–c_2–c_1–h_{21}.

A complete linkage map (Figure 3) for phage P22 has been drawn combining the data from Table 1 with the recombination data from the crosses listed in Table 2. The recombination frequency between the h_{21} and m_4 loci has not been determined directly. The map distance given in Figure 3 is intended only as a rough approximation, derived by the difference between the distance from g to h_{21} and that from g to m_4.

DISCUSSION

All clear-plaque forming mutants of phage P22 can be classified into one of three groups based on the ability to lysogenize. The genetic evidence demon-

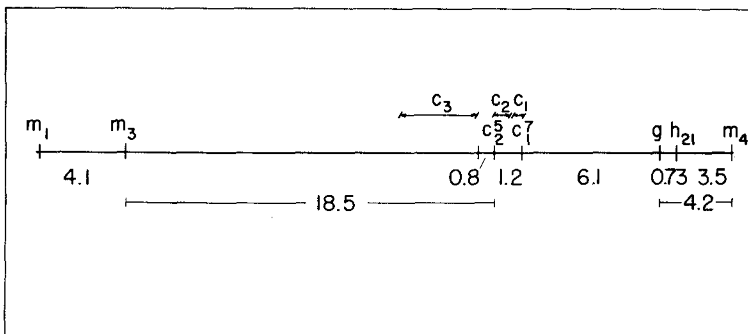

FIGURE 3.—Linkage map for phage P22 mutations. The order and distances between plaque-morphology markers and plaque-morphology markers and c mutations are based on the crosses listed in Table 2 and on the four factor crosses mentioned in the text. The distance between h_{21} and m_4 is only an approximation, derived from the difference between the map distance from g to h_{21} and that from g to m_4. The distances between c mutations are derived from Figure 1.

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TABLE 2

Genetic recombination in the lytic yield of crosses involving plaque morphology mutants

Parents*	Percent recombination										Total progeny
	m_1–m_3	m_3–c_2^5	m_1–c_2^5	c_2^5–h_{21}	m_3–h_{21}	c_1^7–h_{21}	c_1^7–g	g–h_{21}	c_1^7–m_4	g–m_4	
$m_1 + c_2^5 \times + m_3 c^+$	4.1	18.8	21.5	...	...	...	...	...	...	...	994
$m_3 c^+ h_{21} \times + c_2^5 +$	...	18.3	...	7.9	22.1	...	...	...	...	...	1801
$c_1^7 h_{21} \times c^+ +$	...	...	...	...	...	7.0	...	...	...	...	1683
$c^+ g + \times c_1^7 + h_{21}$	...	...	...	...	...	6.4	5.9	0.75	...	...	3893
$g h_{21} \times + +$	...	...	...	...	...	...	...	0.72	...	...	4566
$c^+ g m_4 \times c_1^7 + +$	...	...	...	...	...	...	6.6	...	8.1	3.9	1228
$c_1^7 g m_4 \times c^+ + +$	...	...	...	...	...	...	6.4	...	7.9	4.5	1605

* Parent phages were used at m.o.i. of ten each. The parental factors are given in the order in which they are linked.

strates that the corresponding mutations occur at three closely linked but non-overlapping complex loci or pseudoallelic series which behave like the functional units or cistrons of BENZER (1957). The mutations of each series are separable by recombination into different sites. The alleles of the different loci interact physiologically (complementation) to give lysogeny, whereas the alleles of the same locus do not.

A remarkably similar system of co-operation between clear mutants leading to lysogeny has been reported in coliphage λ (KAISER 1957). Three different phenotypes of clear mutants have been recognized, which fall into three adjacent regions. Mixed infection with a pair of phenotypically different mutants, each of which lysogenizes very poorly or not at all, produces the high frequency of lysogeny typical of the wild type. Clear-plaque forming mutants that show co-operation are also found in phage P2 (BERTANI 1960) and in phage D3 of *Pseudomonas aeruginosa* (EGAN and HOLLOWAY 1961).

It has been proposed (LEVINE 1957) that at least two steps are required for the establishment of lysogeny by phage P22. Phage c^+ can carry out both steps; c_1 mutants are blocked in step 1, c_2 mutants fail in step 2. The phenotypically different mutants each provide, in mixed infection, the function missing in the other, resulting in physiological complementation leading to lysogeny. The c_1 functional unit of phage λ, analogous to the c_2 of P22, determines the locus occupied by the prophage on the chromosome of *E. coli*, the immunity specificity of the phage (KAISER and JACOB 1957) and also controls the production of an immunity substance (JACOB and CAMPBELL 1959). Immunity specificity in phage P22 does not appear to be controlled by the *c* region. ZINDER (1958) has evidence for an immunity locus near the m_3 end of the phage P22 linkage map. The c_2 cistron of this phage may retain some aspect of control of integration of prophage into the bacterial chromosome. This is suggested by the fact that P22 phages carrying c_2 mutations never become prophages (LEVINE 1957). Finally, there are suggestions that the functions carried out by the different functional units may take place at different times after infection (KAISER 1957; TING 1960).

Clear plaque mutants appear among the phages of Serratia (KAPLAN, WINKLER and WOLF-ELLMAUER 1960). In contrast to the phages P22, λ, P2 and D3, no co-operation leading to lysogeny was found in tests among seven X-ray and five ultraviolet-induced *c* mutants of Serratia phage χ. These mutants also show reversions to temperate wild type. Further analysis of the control of temperateness in this phage is clearly indicated.

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SUMMARY

All clear-plaque forming mutants of phage P22 can be classified into three groups; c_1, c_2 and c_3. Genetic fine mapping studies show that the corresponding mutations occur at three adjacent complex loci in which the individual mutations are linearly arranged. A complete linkage map for phage P22 is given.

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