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Genetics of Bacteriophage P22

II. Gene Order and Gene Function

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A complete genetic map of the temperate *Salmonella* phage P22 has been constructed using a variety of methods. The map is circular, about 100 map units (percent recombination) in length, and shows clustering of related functions. The map order by function closely resembles the order reported for the temperate coliphage λ.

INTRODUCTION

Although large numbers of temperate phages are known to exist in nature, only a small number of these have been examined in much detail. Foremost among these is the coliphage λ, which has been studied in great depth over the past two decades. However, it is already quite clear that phage λ represents only one alternative among many with respect to any of its interesting properties. Most other temperate phages which have been studied differ in essential aspects [see Calendar (1970) for review].

The temperate *Salmonella* phage P22 differs in many ways from phage λ. The DNA of λ has cohesive ends (Hershey and Burgi, 1965), whereas phage P22 has linear DNA with circularly permuted sequence and terminal repetitions (Rhoades *et al.*, 1968). λ grows in the absence of recombination (Signer and Weil, 1968), but P22 requires recombination for growth (Botstein and Matz, 1970). λ carries out specialized transduction only, whereas P22 carries out both generalized and specialized transduction (Wing, 1968). λ prophage is repressed completely by a single repressor (Ptashne and Hopkins, 1968; Hopkins and Ptashne, 1971) which completely specifies immunity. P22, on the other hand, uses two gene products to repress the prophage (Gough, 1968) and two separate genetic elements contribute to the specificity of immunity (Bezdek and Amati, 1968; Swanson and Botstein, in preparation). Finally, in order to inhibit superinfection, prophage P22 alters the surface antigens of the host when it lysogenizes (Stocker *et al.*, 1960); in addition, P22 elaborates two superinfection exclusion systems in addition to the bipartite immunity (Rao, 1968; Ebel-Tsipis and Botstein, 1971; Susskind *et al.*, 1971).

For these reasons we have undertaken an extensive study of the biology and genetics of phage P22. The present communication summarizes the current information on the phage P22 genetic map and compares this map with the map of the coliphage λ.

MATERIALS AND METHODS

Phage

Nomenclature. The following nomenclature is used for phages. Genes are numbered as in Gough and Levine (1968). Each mutant allele is preceded by a two-letter phenotype [*ts* = temperature-sensitive, i.e., grows at 25° but not 40°; *am* = amber nonsense mutant; *ug* = UGA nonsense mutant (Lew and Roth, 1970)]. In most cases a letter indicating the mutagen employed (N = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; H = hydroxylamine) follows the phenotype designation.

Often it is convenient to precede the allele designation with the gene number. Thus 20–*am*N106 denotes an *amber* nonsense

mutant, defective in gene 20 isolated after mutagenesis with nitrosoguanidine.

Origin of the phage mutants. Several laboratories kindly supplied mutant phages. Lew and Roth (1970) isolated strains with designations *ug*H1 to *ug*H99; *am*H200-*am*H299. Kolstad and Prell (1969) isolated mutants designated *am*H1-*am*H100: the numbers there correspond to the numbers in their paper (*am*H85 is simply called 85 by them). Gough and Levine (1968) isolated the mutants with decimal notation (i.e., *ts* 10.1); since we use the same gene numbers, these have not been renamed. Levine and his collaborators also isolated mutants designated *ts*N1-*ts*N99 as well as *am*N100-*am*N199. All the other conditional-lethal mutants were isolated in this laboratory.

Other phage. Other phage alleles include the morphological markers c_1^7, c_2^5, m_3, h_{21} (Levine and Curtiss, 1961); *int*3 (defective in prophage integration and called L3 by Smith and Levine, 1967); tsc_2^{29} (Levine and Smith, 1964). The heteroimmune phage L is described by Bezdek and Amati (1967, 1968).

Bacteria

All strains are derivatives of *S. typhimurium* LT2. The prototroph DB21 and the *amber* suppressor strains DB74 (*su*+19) and DB28 (*su*+527-1) and their nearly isogenic *su*− parents DB53 and DB30, respectively, are described in Botstein and Matz (1970). The two suppressors probably do not insert the same amino acid, since some *am* phage strains will grow on only one of them. Strain DB109, which carries both an *amber* suppressor (*su*+19) and a *ug* suppressor was constructed as follows: TR612 [*sup*(UGA)584, *his* deletion 644; kindly supplied by J. Roth], which cannot grow on histidinol since *his*D is deleted, was transduced with *int*3 phage grown on DB30 (*his*C527, an *amber* mutant). A phage-sensitive transductant, able to grow on histidinol (but still auxotrophic) was isolated and purified. This strain was transduced with phage grown on DB74 [*su*+19(*amber*) *his*C527 *cys*A1348] to histidine independence. This strain, when purified, proved to be permissive for *ug* phage mutants, as well as for *am* phage mutants. Although the strain grows somewhat poorly, it transmits wild-type, *am*, and *ug* alleles approximately equally in mixed infections.

The prophage deletion strains and their use in mapping are described in the companion paper (Chan and Botstein, 1972).

Propagation and Assay

The media used are described in detail by Botstein and Matz (1970) or in the companion paper (Chan and Botstein, 1972). Phage stocks are grown from single plaques suspended in 30 ml of LB broth together with 0.5 ml of an overnight culture of an appropriate permissive host. The mixture is shaken at room temperature for 6–24 hr. Some mutants give stocks with much lower revertant frequencies if short times are used. The stocks are concentrated 15-fold by centrifugation and stored at 4° in buffered saline (0.85% w/v NaCl in 0.066 *M* phosphate buffer at pH 7) over chloroform. Titers are stable (within a factor of 2) for at least 2 years.

Assays are done by standard methods (Adams, 1959). Preadsorption of phage before plating is not necessary, nor is starvation or concentration of bacterial cultures before use as indicators. Indicator bacteria are made fresh daily by growing in broth to 5×10^8 cells/ml and chilling.

Complementation Tests

Spot tests. Spot tests for complementation are quite reliable if done at several phage concentrations. The test is done with one of the mutant phages, plated together with the appropriate nonpermissive indicator. The other parent is spotted onto the plate with a sterile capillary tube. Three concentrations of each mutant phage are prepared (10^8, 10^7, and 10^6 per milliliter) in buffered saline. A plate is prepared for each concentration of each phage to be tested (0.1 ml of phage suspension and 0.2 ml of indicator in 2 ml of top agar). As soon as the agar hardens, the three concentrations of each phage to be tested are spotted, and the plate is allowed to dry and is immediately incubated at 37°. If *ts* mutants are involved, the plate is rapidly warmed on a hot plate to 40° just before spotting. A spot of wild-type (c_2) phage is included on each plate.

Failure to show growth in this test is a reliable indication of failure to complement. The method tests each mutant pair twice, once with each mutant in the background; both of these tests should agree. Growth in the test can be misleading, since intragenic recombination frequencies in some genes can be as high as 10%.

Complementation in liquid culture. These tests are carried out between every mutant and at least one tester mutation in the same gene as well as at least one tester in a neighboring gene. In some genes recombination frequencies are very high and spot tests are nearly useless; liquid tests have been carried out on many combinations of mutants in these genes.

The method is exactly the same as the standard cross (see below) except that an appropriate nonpermissive host is used. In cases involving *ts* mutants, the test is carried out at 40°. In most cases a standard cross in a permissive host is done simultaneously, using the same mixture of phage. A burst size in the complementation test of less than 2 phage per cell signifies no complementation. When complementation occurs, the burst sizes are at least 10 phage per cell. If one or both of the mutants being tested is c^+, the complementation burst sizes are quite low (10–30 phage per cell); this effect is seen also in the permissive crosses. Sometimes c_1 or c_2 derivatives have to be constructed in order to test complementation unambiguously. Most *ts* and some nonsense mutants have also been tested in nonpermissive mixed infections with wild-type (c_2) phage. This is necessary in order to rule out the possibility of dominance or "negative" complementation. A few cases of dominance have been observed with *ts* mutants, but never with nonsense mutants.

Complementation for lysis mutants. Since mutants in genes 19 and 13 produce at least normal numbers of viable phage under nonpermissive conditions but the cells fail to lyse, standard liquid complementation tests often gave anomalous results. Complementation was carried out in these cases for cell lysis by coinfecting two mutants under nonpermissive conditions at high cell density (2×10^8 cells/ml) and following optical density in a Klett-Summerson colorimeter.

Standard Cross

The method described by Botstein and Matz (1970) is used with very slight modification. Cells are grown in M9CAA (a minimal medium containing amino acids) to 6×10^8 cells/ml, centrifuged in the cold, resuspended in an equal volume of buffered saline, and infected with 7 phage/cell of each parent. After 15 minutes at 25°, the complexes are diluted 10^4-fold and allowed to incubate for 3 hr at 25°. Chloroform is added and the progeny are assayed for total yield and numbers of recombinants.

RESULTS

Mutant Isolation and Complementation

A summary description of the conditional-lethal mutants which have been characterized to date is presented in Table 1. Several laboratories have contributed temperature-sensitive (*ts*), amber (*am*), and UGA nonsense (*ug*) mutants isolated by a variety of standard methods.

The isolation methods had a noticeable effect on the gene distribution of mutants obtained. The mutagenesis method seemed not to have any marked effect, except that nitrosoguanidine mutants tended to have secondary mutations and occasionally had to be discarded in the end.

The mutants were classified into complementation groups using spot tests. Spot tests are usually reliable, but only if several phage concentrations are tested at the same time. In some cases, spot tests were nearly useless, because the frequency of recombination between different alleles in the same gene can be as high as 10%. Complementation tests by coinfection under nonpermissive conditions had to be carried out in these cases.

When a large number of prophage deletion strains became available (see the preceding paper: Chan and Botstein, 1972), it became more convenient to map mutants before attempting complementation tests. Mutants classified in this way had only to be tested in liquid-culture complementation tests with a few nearby genes. However, all mutants

TABLE 1
MUTANTS IN ESSENTIAL GENES AND THEIR PHENOTYPES

Gene	Number of alleles			DNA synthesis	Lysis	Base-plate activity	Viability and morphology
	ts	*am*	*ug*				
1	2	12	1	+	+	+	Partially empty heads
2	2	3	0	+	+	+	Partially empty heads
3	1	1	4	+	+	+	Partially empty heads
5	7	8	1	+	+	+	Nothing visible
6	1	0	0	±[a]	−	−	—
8	0	8	0	+	+	+	Aberrant empty heads
9	8	3	1	+	+	−	Viable tailless heads
10	4	5	2	+	+	+	Empty heads
12	3	5	0	−[b]	−	−	—
13	0	2	0	+	−	+	Viable phage
16	0	2	0	+	+	+	Inviable phage
18	2	0	0	−[b]	−	−	—
19	2	3	0	+	−	+	Viable phage
20	0	8	3	+	+	+	Inviable phage
21	1	0	0	±[a]	−	−	—
23	0	2	0	NT[c]	−	NT	—
25	2	0	0	−[a]	−	−	—
26	0	2	0	+	+	+	Empty heads

[a] Incorporation measurements; ± means leaky phenotype.
[b] Density transfer experiments. Mutants in these genes are described further by Levine and Schott (1971).
[c] NT = Not tested.

TABLE 2
COMPLEMENTATION OF GENE 5⁻ AND GENE 10⁻ MUTANTS[a]

Gene	Allele	*am*N8*c*⁺	*ts*N15*c*₂	*ts*N13*c*₂	*ts*5.1*c*₂	*am*N13*c*⁺	*am*N3*c*⁺	*ts*N8*c*₂	*ts*10.1*c*₂
5	*am*N8*c*⁺	<0.01							
	*ts*N15*c*₂	0.5	0.06						
	*ts*N13*c*₂	0.1	2	0.2					
	*ts*5.1*c*₂	0.02		0.2	0.02				
	*am*N13*c*⁺	0.6	0.17		0.05	<0.01			
	*am*N3*c*⁺	0.14				0.03	<0.01		
10	*ts*N8*c*₂	18	41	52	8	83	32	0.2	
	*ts*10.1*c*₂	20	98	81	1	39	7	0.2	0.2
	wild-type *c*₂	27	270	100	3.2	64	24	150	51

[a] Burst sizes are given as phage released per infected cell. Multiplicity of infection was 7 of each phage. The nonpermissive host was DB21; all the experiments were done at 40°.

were tested against representatives of many other genes and wild-type using the spot tests. As shown in Table 1, the complementation analysis yielded 18 groups, two of which contain only one member.

Except for the recombination problem discussed above, very few complementation anomalies were observed. The most important of these involves a few temperature-sensitive mutants. Several of these (5⁻*ts*5.1, 9⁻ *ts*N16, 25⁻ *ts*25.1 and to a lesser degree 18⁻ *ts*18.1) depress the yield of wild-type both in spot tests and in liquid nonpermissive coinfection. Table 2 shows this effect of *ts* 5.1. The table also shows a typical sample of the data obtained from complementation tests in liquid.

The table also illustrates the effect of

lysogenization on the complementation tests. Since the tests are carried out at a total multiplicity of infection of 10–20 phage per cell, lysogeny is favored if one or both of the infecting phages is c^+ (Levine, 1957). The burst is quite small in these cases and can be misleading if not properly controlled. Lysogenization does not affect spot tests as much and has no effect on recombination frequencies in permissive crosses.

Mutant Phenotypes

A detailed study of the various mutant phenotypes is in preparation. However, a brief summary of the results is included in Table 1. Mutations in 5 of the 18 known genes (genes 6, 12, 18, 21, and 25) have an "early" phenotype by the criterion that little or no DNA is synthesized and the infected cells do not lyse. A sixth gene (gene 23) has not yet been tested for DNA synthesis, but could fall into this group, since the infected cells fail to lyse and no phage are produced.

Mutants in two genes (13 and 19) are defective in lysis but produce a large burst of viable phage which can be liberated by artificial lysis methods. Gene 19 mutants synthesize altered lysozyme or none at all. Gene 13 mutants have a phenotype similar to mutants in gene S of the coliphage λ (Goldberg and Howe, 1969; Reader and Siminovitch, 1971): they fail to lyse and continue to produce viable phage for many hours beyond the normal lysis time.

There are 10 other "late" genes. Mutants in one of these (gene 9) produce full phage heads with no baseplate; these can be reconstituted into viable phage using partially purified base plate protein (Israel *et al.*, 1967). The remaining genes are involved in phage head synthesis, DNA maturation, or both. Mutants in all of these produce base plate protein which can activate tailless phage heads. Mutants in all genes except gene 5 produce structures recognizable as some kind of head membrane in the electron microscope: by a variety of criteria gene 5 appears to code for the major head structural protein. Finally, mutants in two late genes (16 and 20) produce morphologically normal (full) but inviable phage.

TABLE 3
PHENOTYPES OF MUTANTS IN NONESSENTIAL GENES

Gene	Function affected	References
c_1	Establishment of lysogeny	Levine (1957), Smith and Levine (1964)
c_2	Establishment and maintenance of lysogeny	Levine (1957), Smith and Levine (1964)
c_3	Establishment of lysogeny	Levine (1957); Smith and Levine (1964)
mnt	Maintenance of lysogeny	Gough (1968)
erf	Recombination (essential to growth in *rec*⁻ host)	Botstein and Matz (1970)
int	Prophage integration	Smith and Levine (1967), Smith (1968)
*sie*A	General superinfection exclusion	Rao (1968), Ebel-Tsipis and Botstein (1971), Susskind *et al.* (1971)
*sie*B	Superinfection exclusion heteroimmune phages only	Susskind *et al.* (1971)
K5	Component of virulence constitutive, (new promoter?)	Bronson and Levine (1971)
Vx	Component of virulence	Bronson and Levine (1971)
Vy	Virulence	Swanson and Botstein, in preparation.
m_3	Plaque morphology	Levine (1957); Levine and Curtiss (1961)
h_{21}	Plaque morphology	Levine (1957); Levine and Curtiss (1961)

A substantial number of P22 genes which are not essential to growth in wild-type *Salmonella typhimurium* have also been identified and mapped. The phenotypes of their mutants are briefly outlined in Table 3. In this class fall the clear-plaque mutants c_1, c_2, and c_3. Gene c_2 specifies a P22 repressor required for the maintenance of

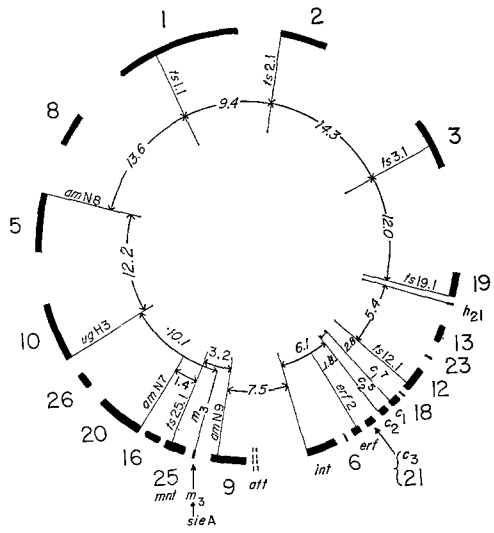

FIG. 1. The genetic map of phage P22 drawn approximately to scale. The genetic size of each gene is indicated; this was determined by the largest frequency of recombination between mutations in a complementation group. Also shown are frequencies of recombination between particular mutants in the various genes; these values have been normalized to the interval $c_1 - h_{21} = 6.8\%$ recombination determined in the same cross (Gough and Levine, 1968). The total length of the map is 100% recombination (map units).

lysogeny (Levine and Smith, 1964) whereas the products of the c_1 and c_3 genes are required only for establishment of repression, and thus lysogeny. A second gene, *mnt*, also specifies a repressor which is required for the maintenance of lysogeny (Gough, 1968). The *int* gene (Smith and Levine, 1967; Smith, 1968) is required to integrate the prophage and thus in the establishment of lysogeny. The *erf* gene specifies part of a general recombination system (Botstein and Matz, 1970); *erf*$^-$ mutants are conditional lethal in the sense that they grow in *rec*$^+$ but not *rec*$^-$ hosts. The *sie*A and *sie*B genes specify components of systems which the prophage elaborates to prevent superinfection. These are different from and act independently of prophage immunity (Rao, 1968; Ebel-Tsipis and Botstein, 1971; Susskind *et al.*, 1971). Three kinds of mutations involved in virulence [capacity to grow on a nonexcluding (*sie*A$^-$) lysogen] are known. K5 and Vx map in the c_2 region; only Vx K5 double mutants (called *vir*B) are virulent (Bronson and Levine, 1971). Vy mutants map in the *mnt* region and are virulent (*vir*A) by themselves (Bronson and Levine, 1971; Swanson and Botstein, in preparation). Finally the plaque morphology markers m_3 and h_{21} (Levine, 1957; Levine and Curtiss, 1961), which have been so useful in mapping the early region of the phage map, have no known effect on phage growth, save the appearance of the plaques on green indicator plates.

The Genetic Map

A comprehensive genetic map of phage P22 is shown in Fig. 1. The genetic sizes of the genes are represented by the length of the bars; their values as well as the distances between genes are determined from recombination frequencies in vegetative phage crosses. The total map length is about 100 recombination units. The relation of these units to physical distance is not known; however, gene order in almost every case has been confirmed using prophage deletions (Chan and Botstein, 1972). In two cases (c_3 and gene 21; *mnt*, *sie*A, and gene 25) the gene order is not certain.

The vegetative genetic map of phage P22 is circular (Gough and Levine, 1968) due to the circular permutation and terminal repetition in the phage DNA (Rhoades *et al.*, 1968). The prophage map, on the other hand, is linear with unique ends. The circular map is broken at the point marked *att* in Fig. 1, as expected if the Campbell model (Campbell, 1962) for insertion of the prophage were to apply to P22. The attachment site (*att*; the ends of the prophage) was first located by Smith (1968). His result has been confirmed and extended by the isolation and characterization of prophage deletions, as described in the preceding paper (Chan and Botstein, 1972).

In the following sections, the basis for the map in Fig. 1 will be presented. Regions of the map will be considered in detail, beginning with the attachment site and proceeding counterclockwise.

The region att-c_1. The shortest proline deletion begins in the *pro*A gene of the host and extends into or beyond the c_1 gene. It deletes *int*, gene 6, *erf*, gene 21, c_3, c_2, and

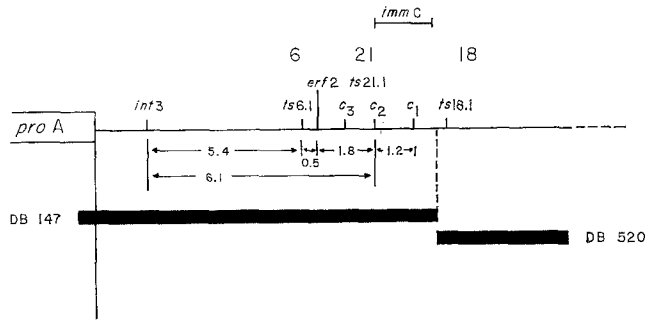

FIG. 2. Gene order in the region *att*-c_1 . The recombination frequencies are normalized as in Fig. 1. The heavy bars indicate the genetic material deleted in prophage deletion strains (Chan and Botstein, 1972). The bar marked *imm*C indicates the approximate extent of the region of nonhomology between phages P22 and L in this part of the map. The numbers near the top indicate gene number; the particular alleles used in mapping are indicated beneath them.

all or part of c_1 (Fig. 2). The *int* gene was located between gene 9 and c_2 by Smith (1968). Table 4 shows the results of a cross between *int*$_3$*erf*$_2$ and *ts*6.1 (gene 6). This cross shows that *int* is to the left of gene 6. Recombination frequencies (about 6% for *int*-c_2 and only 2% for 6-c_2) support this order.

The order 6-*erf*-21-c_2 was demonstrated by Botstein and Matz (1970). The order c_3-c_2-c_1-h_{21} was shown by Levine and Curtiss (1961). These two sections can be oriented with respect to each other since deletions exist which delete the entire segment *att*-c_1 but do not delete h_{21} .

Gene order in this region is therefore *att*-*int*-6-*erf*-(21-c_3)-c_2-c_1-h_{21} , illustrated in Fig. 2, which also presents data concerning map distances between these genes. The order of c_3 and gene 21 has not been definitely established, although recombination frequency data (not shown) suggest the order *erf*-21-c_3-c_2 .

The immunity of phage P22 is specified by two regions: exchange of either with the heteroimmune phage L changes the immunity specificity of the resultant recombinant phage (Bezdek and Amati, 1968). Figure 2 shows the extent of one of these regions named *imm*C (this corresponds to ^{P}C of Bezdek and Amati). The wild-type alleles of *ts*18.1, *ts*12.1, *ts*21.1, and *erf*2 can be rescued from phage L by recombination without acquisition of a new immunity specificity. This implies that genes 18, 12, 6, and *erf* all lie outside the region which specifies *imm*C of P22. On the other hand, Bezdek *et al.* (1970) have shown that c_3 alleles, but not c_1 or c_2 alleles, can be exchanged between phages P22 and L without exchange of the immunity specificity. Therefore the region of difference between *imm*C_{P22} and *imm*C_L must begin somewhere between genes (c_3 , 21) and c_2 , and end between genes c_1 and 18.

The region c_1-h_{21} . This region has been mapped in two ways. Table 5 contains the result of a series of 4-factor crosses which establish the order (c_1 , c_2)-18-12-23-13-h_{21} . Each cross selects recombinants between two *ts* or *am* markers and follows the linkage of unselected plaque-morphology markers. In most cases the reciprocal crosses were also done, with the appropriate results. Similar results are reported by Levine and Schott (1971). The set of deletions shown in Figure 3 also enabled unambiguous determination

TABLE 4
*int*3 *erf*2 × 6$^-$*ts*6.1[a]

Selected markers: *ts*$^+$*erf*$^+$		int + erf + ts +
Unselected marker	Number	Crossover class
int	312	Single
int$^+$	108	Double

[a] Standard cross carried out in DB21. Recombinants were selected on DB47 (*rec*$^-$) at 40°. The integration phenotype was scored by the method of Smith and Levine (1967) on green indicator plates in strain DB21.

TABLE 5
ORDER IN THE REGION c_1 – h_{21}[a]

1. 12$^-$*ts*12.1 × 18$^-$*ts*18.1 $m_3c_2h_{21}$ Selected markers: ts^+

c 18 + h
— / —
+ + 12 +

Unselected markers	Number	Crossover class
+ +	131	Double
c h	46	Double
+ h	423	Single
c +	16	Triple

Order deduced: c_2–18–12–h_{21}
Frequency of recombination *ts*18.1–*ts*12.1 = 2.3%

2. 12$^-$*am*N14 c_1h × 23$^-$*am*H79 Selected markers: am^+

c 12 + h
— / —
+ + 23 +

Unselected markers	Number	Crossover class
+ +	107	Double
c h	31	Double
+ h	466	Single
c +	11	Triple

Order deduced: c_1–12–23–h_{21}
Frequency of recombination *am*N14–*am*H79 = 4.0%

3. 23$^-$*am*H79 c_2h × 13$^-$*am*H715 Selected markers: am^+

c_2 23 + h
— / —
+ + 13 +

Unselected markers	Number	Crossover class
+ +	30	Double
c h	115	Double
+ h	453	Single
c +	8	Triple

Order deduced: c_2–23–13–h_{21}
Frequency of recombination: *am*H79–*am*H715 = 3.4%

[a] Standard crosses were carried out in DB21 (cross 1) and in DB74 (cross 2 and 3). The reciprocal crosses with the *c* and *h* markers on the opposite parent were carried out for both crosses 1 and 2 with the expected results.

of the same map order. The h_{21} allele was mapped between genes 19 and 13 by examining progeny of a single-cycle of growth of 19$^-$*ts*19.1 h_{21} on the deletion strain DB5066, in which a part of gene 19 is deleted. About half the ts^+ progeny are also h^+; h^+ is therefore separable from *ts*19.1 and not deleted in DB5066 (Fig. 3). A similar experiment (23$^-$*am* H79 h_{21} grown on DB5208) shows no h^+ progeny; h^+ is presumably deleted in this strain. Furthermore, cross 3 in Table 5 shows that h_{21} is to the right of 13$^-$*am*H715. Thus the order of this segment must be c_1-18-12-23-13-h_{21}-19, as shown in Fig. 3. It is possible that the h_{21} mutation may be within gene 19 or gene 13. An alteration in a gene specifying a lysis function can easily be imagined to alter the plaque morphology.

The region gene 19-*gene* 10. This region was mapped by deletion mapping as described in the preceding paper (Chan and Botstein, 1972). It is noteworthy that many of the genes in this region are between 6 and 11 recombination units long. Some of the distances between genes (Fig. 1) are taken from Gough and Levine (1968); crosses in our laboratory give frequencies indistinguishable from those reported by them.

The region gene 10-*att*. The order gene 10-26-20-16-(25, 9) was established by the deletions shown in Fig. 4. However, since the deletions could not distinguish the orientation of genes 25 and 9 with respect to the rest of the map, a three-factor cross (Table 6) was done which places gene 25 between genes 5 and 9.

One of the two regions which specify P22 immunity, *imm*I, is located in this region. In the preceding paper (Chan and Botstein, 1972) it was shown that $imm\mathrm{I_P}$ cannot be rescued from the longest *pro*$^-$ deletion strain (DB136) although it is rescued from all the other *pro*$^-$ deletions. Furthermore, $imm\mathrm{I_P}$ cannot be rescued from any of the tet^R deletion strains, including the shortest one (DB5057). These results place *imm*I between genes 20 and 25 (Fig. 4).

In order to place *imm*I more precisely, a series of crosses was carried out to determine whether particular conditional-lethal markers could be separated from $imm\mathrm{I_L}$. As shown in Table 7, alleles of genes 10, 20, 16, and 9 can be rescued from an L lysogen under permissive conditions without the concomitant acquisition of $imm\mathrm{I_L}$. However, in the case of 25$^-$*ts*25.1, the frequency with which $imm\mathrm{I_P}ts^+$ progeny arise in such an experiment is not significantly greater than the reversion frequency. These results

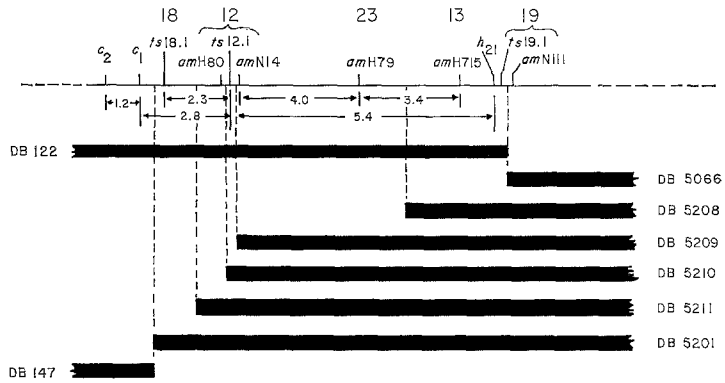

FIG. 3. Gene order in the region c_1-gene 19. Notation as in Fig. 2.

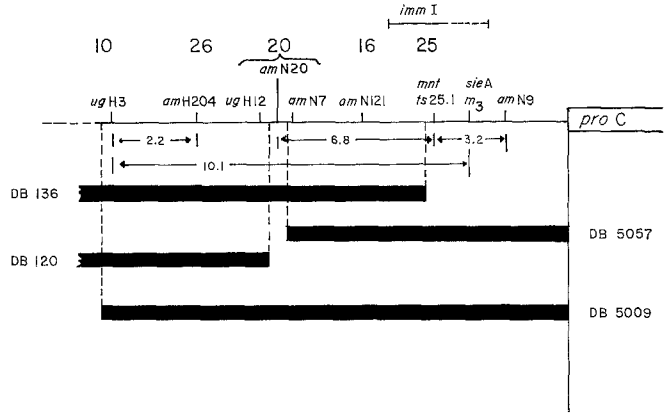

FIG. 4. Gene order in the region gene 10-*att*. Notation as in Fig. 2. The bar marked *imm*I indicates the approximate extent of the region of nonhomology between phages P22 and L in this part of the map; it is not yet clear (see text) whether the markers under the dashed part of the bar are within *imm*'I

suggest that all or part of gene 25 is within the region of nonhomology between phages P22 and L at *imm*I.

The plaque-morphology marker m_3 (Levine, 1957) and the superinfection-exclusion gene *sie*A (Susskind *et al.*, 1971) lie within or very near *imm*I. In numerous crosses between P22 and L, $imm\mathrm{I}_\mathrm{L}m_3$ or $imm\mathrm{I}_\mathrm{L}$*sie*A recombinants have never been observed. This means little, however, since no selection could be applied and it is not certain that either the m_3 or *sie*A phenotype can be expressed in $imm\mathrm{I}_\mathrm{L}$ phages.

Both *sie*A and m_3 can be rescued from the longest proline deletion (DB136) and neither can be rescued from the shortest tetracycline deletion (DB5057) (Fig. 4; Susskind *et al.*, 1971). Thus *sie*A and m_3 must lie within or to the right of *imm*I, since DB136 deletes part of *imm*I.

We attempted to map *sie*A and m_3 more precisely by four-factor crosses involving genes 9, 25, and 20 (Table 8). The first cross shows that both m_3 and *sie*A are closer to gene

TABLE 6
MAP ORDER OF GENES 5, 25, AND 9[a]

Cross	Burst size	Frequency of ts^+ recombinants (%)
*ts*25.1 × *ts*9.1	37	1.3
*ts*25.1 × *ts*N13	47	5.7
*ts*25.1 × *ts*9.1 *ts*N13	40	0.28
Order deduced: *ts*N13–*ts*25.1–*ts*9.1		
Interference index = 2		

[a] *ts*25.1, *ts*9.1, and *ts*N13 are in genes 25, 9, and 5, respectively. The crosses were standard crosses at m.o.i. of 7 of each parent.

TABLE 7
LOCATION OF *imm*I

Gene	Allele	B clear ts^+ or am^+ among all progeny	A $imm I_{P22}$ among clear ts^+ or am^+	A × B $imm I_{P22}$ clear ts^+ or am^+ among all progeny	Revertants
10	*am*N107c_1	6.5×10^{-2}	130/200	4×10^{-2}	2×10^{-7}
20	*am*N20c_2	8×10^{-3}	21/196	9×10^{-4}	2×10^{-7}
16	*am*N121c_1	2×10^{-2}	8/96	2×10^{-4}	3×10^{-7}
25	*ts*25.1c_1	2×10^{-3}	1/550	$\sim 4 \times 10^{-6}$	1×10^{-6}
9	*ts*N16c_2	3×10^{-4}	24/24	3×10^{-4}	2×10^{-6}

25 than to gene 20. In addition, the segregation pattern weakly suggests the order 20-25-(*sie*, m_3). This order is strongly supported by the second cross. Here its obvious that *sie*A is closely linked to gene 9, and that m_3 is less strongly linked to gene 9. The segregation here all but excludes the possibility that either m_3 or *sie*A is to the left of gene 25, since this would demand that the predominant class (sie^+m^+) among am^+ts^+ progeny be a double-crossover.

Location of the gene controlling the phage-specified surface antigen (factor 1). The gene for the P22-specified surface antigen has been mapped using the prophage deletions. Strains carrying the longest proline deletion (DB136) are precipitated by factor 1-specific antibody, as are L lysogens. Therefore we presume that the gene(s) specifying factor 1 lie between the right-hand end of *imm*I and the *att* site. This determinant had been placed between m_3 and c_2 by Young *et al.* (1964).

Thus, this region of the map is ordered as follows: 10-26-20-16-25-m_3-(*sie*A-9)-*att*. *Imm*I begins between gene 16 and m_3; it probably includes gene 25 and could include m_3 and *sie*A as well.

It should be emphasized that the placement of *sie*A and m_3 is less certain than all the other mapping; the relevant recombinant phenotypes are sometimes unknown and sometimes difficult to score. Definitive mapping awaits the isolation of deletions lying within the region *25-att*.

TABLE 8
LOCATION OF m_3 AND *sie*A[a]

1. 20⁻*am*N7m_3*sie*1 × 25⁻*ts*25.1
Selected markers: ts^+am^+

Unselected markers		Number
m_3	*sie*A1	162
+	*sie*A1	66
m_3	+	73
+	+	43

sie^+: 116/337 = 35%
m^+: 109/337 = 32%
Frequency of recombination *am*N7-*ts*25.1 = 1.4%
Probable order: 20-25-(*m*, *sie*A)

2. 9⁻*am*N9m_3*sie*1 × 25*ts*25.1
Selected markers: ts^+am^+

Unselected markers		Number
m_3	*sie*A1	13
+	*sie*A1	24
m_3	+	133
+	+	320

sie^+: 453/490 = 92%
m^+: 344/490 = 70%
Frequency of recombination *am*N9-*ts*25.1 = 3.2%
Probable order: 25-*m*-(9, *sie*A)

[a] Standard crosses were carried out in DB74.

DISCUSSION

The present genetic map of phage P22 (Fig. 1) is the sum of many different kinds of experiments. Most of the theoretical background for complementation, vegetative crosses, marker rescue experiments, and deletion mapping is standard. However, in the application of these principles to the genetics of phage P22 we have encountered several points of interest, and we wish to sound some notes of caution. These are summarized below.

Complementation

Before prophage deletions became available, several erroneous assignments of

mutants to complementation groups had been made. Several of these were assigned not only in spot tests, but also on the basis of complementation tests carried out in liquid medium. Spurious noncomplementation is easily recognized from the results of mixed infection of mutant and wild-type in a nonpermissive host. As shown above, some temperature-sensitive alleles are dominant, and therefore interfere with growth of all phage in the complementation test. These cases are rare, and all were immediately recognized from the wild-type controls.

Apparent complementation among mutants which belong in the same cistron was a much more serious problem, however, which accounted for all of our erroneous assignments as well as those in other laboratories. Examination of these cases in detail showed that the large bursts of phage obtained after coinfection of mutants in a nonpermissive host are due to recombination. This was demonstrated by measuring the number of recombinants in such a burst and comparing this with the frequency of recombinants in a permissive cross. In these cases, the frequency of wild-type recombinants in the nonpermissive host was much higher and often exceeded 25% of the burst, which is the theoretical limit. This result is to be compared with genuine complementation, where the frequency of recombinants is within a factor of 2 of normal. This effect was most noticeable in late genes; in particular, gene 1 was divided into two groups by several laboratories. The most effective remedy for this difficulty turned out to be to do the complementation tests in a *rec*$^-$ host; the 2-fold reduction in recombination (Botstein and Matz, 1970) is apparently adequate. It is a good practice to measure the number of recombinants in every complementation test. After some mapping data became available, we often carried out complementation tests in liquid between the nearest mutants in neighboring genes, in order to be certain that these are in fact different genes. In other instances, differences in phenotype sufficed to confirm the assignments.

By far the most reliable, as well as the least laborious method for establishing gene order is the determination of the presence or absence of phage alleles in prophage deletion strains (Chan and Botstein, 1972). This method works as well as it does primarily because of the high rate of recombination among P22 genomes in P22-infected cells: markers are rescued efficiently even if the marker and the deletion end point lie within the same cistron.

Multifactor crosses in which nonselected markers flank the alleles whose order is to be determined are nearly as useful as deletion mapping in the ascertainment of order. This method has been used extensively with plaque-morphology markers (m_3, h_{21}, c_1 or c_2; Table 5). In our hands this method is reliable if the total distance under examination is less than about 10 map units and if reciprocal crosses are performed. In one or two cases this method has failed because the phenotype of a plaque-morphology marker is modified by conditional-lethal mutations even under permissive conditions. For example, some mutants in gene 9 will not express an m_3 marker which they carry. Simple three-factor crosses are also useful (Tables 4 and 6); negative interference has not been a major problem in mapping P22.

Recombination frequency alone is useful as a mapping tool when all crosses are normalized to a standard interval (Gough and Levine, 1968). In order to construct Fig. 1, we normalized to the interval c_1-h_{21} = 6.8%. The actual measurement varies over the range 5% to 9%. Normalization minimizes variation due to total multiplicity, lysis time, etc., in the crosses. It should be emphasized that none of the gene order in our map depends on recombination frequency alone. The total length, after normalization, of the circular genetic map is almost exactly 100 map units. Individual genes can reach a size of 11 map units (gene 1), or about 11% of the total map.

The only other method which seems to be of general use in mapping phage P22 is the determination of linkage of markers during marker rescue from a prophage deletion or from a heteroimmune lysogen. This method enabled us to locate gene 25 relative to *imm*I (Table 7).

TABLE 9
NEGATIVE INTERFERENCE AS A FUNCTION OF MAP INTERVAL[a]

Markers	Recombination over total interval (%)	Interference index
c_2^5–18⁻*ts*18.1–12⁻*ts*12.1	3.3	8.3
18⁻*ts*18.1–12⁻*ts*12.1–h_{21}	7.5	4.4
c_2^5–12⁻*am*N14–23⁻*am*H79	5.8	3.8
5⁻*ts*N13–25⁻*ts*25.1–9⁻*ts*9.1	14.0	2.0

[a] Recombination frequencies are taken from the cross and not normalized.

Negative Interference

Although the normalized map distances are roughly additive over the range 2–20%, P22 shows high negative interference. The magnitude of this effect is comparable to that found by Chase and Doermann (1958) for phage T4 and Amati and Meselson (1965) for phage λ. Table 9 contains some representative values of the interference index (derived from the data in Tables 5 and 6). It is clear that negative interference is strongest in crosses involving the shortest total intervals, as found for phages T4 and λ (Chase and Doermann, 1958; Amati and Meselson, 1965).

Correlation with Other Maps of Phage P22

The map in Fig. 1 is entirely consistent with that of Gough and Levine (1968). Both maps are circular, of approximately equal length (90 vs 100 units), and the gene order is in every case the same. The only discrepancy concerns the mutant *ts* 11.1. While we could repeat Gough and Levine's crosses with comparable results, we could not place *ts* 11.1 among the deletions. It became clear that *ts* 11.1 in fact consists of two linked mutations.

The other published map of phage P22 is that of Kolstad and Prell (1969). We have examined representative mutants from their collection which were kindly provided by Dr. Kolstad. We find the following correspondence (in map order) between their complementation groups and ours. Kolstad and Prell group 80 is gene 12; group 79 is gene 23; group 62 is gene 19; groups 58 and 21 are both gene 1 (illustrating the difficulties of complementation in the presence of high levels of recombination); group 69 is gene 5; group 70 is gene 10; group 85 is gene 26; group 64 is gene 20, and group 60 is gene 16. The map order is the same in most cases, as are the relative genetic distances. The only discrepancy concerns the location of mutants 79 and 80 relative to c_2 and the rest the map. Kolstad and Prell (1969) give the order 80-c_2-79-62. We find the order to be c_2-80-79-62 (i.e., c_2-gene 12-gene 23-gene 19 in our notation). We have confirmed the correct order by deletion mapping (Fig. 3) and by complementation in liquid for each of the mutants given to us as well as by four-factor crosses (Table 5). It should be emphasized that the misplacement of 12⁻*am*H80 (Kolstad and Prell mutant 80) is the only error in their map. Unfortunately this error was propagated by Kolstad (1971), who showed that *am*H80 was in gene 12 and then used the erroneous relation to c_2 to align his map with that of Gough and Levine (1968).

Functional Organization of the P22 Genetic Map.

The genetic map of phage P22 (Fig. 1) shows a very high degree of functional organization. Genes specifying related functions are clustered. This clustering not only separates genes acting early from those acting late in infection, but extends even to the details of phenotype. For example, the mutant phenotypes of mutants in the neighboring genes 20 and 16 are the same: encapsulated, morphologically normal, but biologically defective phage are produced. Similarly, the adjacent genes 10 and 26 apparently fail to stabilize heads after encapsulation, and defects in the adjacent genes 1, 2, and 3 result in the failure to encapsulate DNA and in the production of morphologically indistinguishable head precursor structures. Genes 19 and 13 govern lysis and are neighbors; genes 18 and 12, also neighbors on the map, are both involved in DNA replication.

The only major exception to clustering concerns the regions specifying immunity

and repression. These functions are split into two regions: one (*imm*C) near the DNA replication genes in the "early region" and the other (*imm*I) in the "late region" between the head and tail genes.

The reason for this clustering of P22 genes specifying related functions (and for the exceptions) is not known: the direction of transcription has not been unambiguously established in any case; nor has clear evidence for operons or other units of control been reported. Nevertheless the c_2 and *mnt* repressors located in *imm*C and *imm*I regions, respectively, must control, directly or indirectly, all phage functions in a lysogen. The arrangement of the genetic map, especially the separation of *imm*I and *imm*C from each other and the separation of gene 9 from the other late genes suggest that P22 will be found to have a complicated and probably novel system of control of gene expression.

Comparison with Coliphage λ

Despite the manifold differences between phage P22 and phage λ, the maps of the two phages are strikingly similar. There is almost a one-to-one, gene-for-gene identity in the order of genes classified by function (Fig. 5). The major differences are the P22 *imm*I region for which no λ analog exists, and the λ gene N, for which no analogous P22 gene is known. The similarity between the maps is puzzling, if one remembers that head maturation must be very different in these phages, due to the differences in the mature DNA form [linear with cohesive ends (λ) and circularly permuted and terminally repetitious (P22)]; that the tails of the two phages are radically different; that immunity is different; and that even the relation of DNA replication to lysogeny is different [P22 requires DNA replication (Levine and Schott, 1971); λ does not (Signer, 1968)].

The similarity in the P22 and λ maps could be accounted for by a similarity in mode of control or by a common evolutionary ancestry. The latter possibility is supported by the fact that P22 and λ share some 18% sequence homology according to hybridization experiments (Cowie and Szafranski, 1967). Most of this homology lies in the right arm of λ (Skalka and Hanson, 1972), where the similarity between the phages, both genetically and functionally, is most exact. A further discussion of these issues is to be found in Dove (1971).

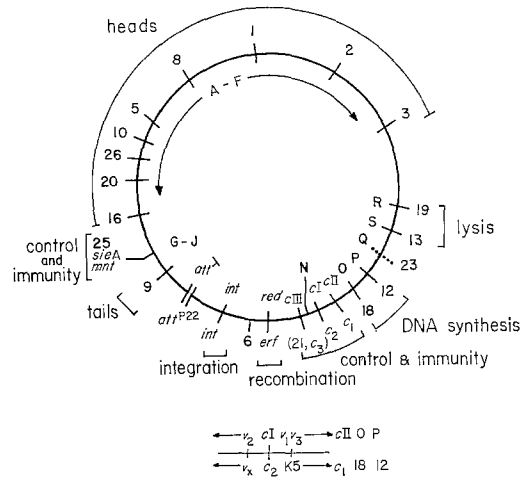

FIG. 5. Comparison of the maps of phage P22 and coliphage λ. P22 is drawn to approximate scale; λ is not. The inside of the circle shows the λ map and the outside gives the P22 map. The information for P22 comes from Fig. 1 and Tables 1 and 3; the order and functions of λ genes is taken from Campbell (1971). A solid line connecting markers of the two phages indicates substantial similarity in gene function. At the bottom are shown constitutive mutations which together produce virulence in each of the two phages (Bronson and Levine, 1971).

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