THE CIRCULARITY OF THE PHAGE P22 LINKAGE MAP[1,2]

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Received September 26, 1967

PHYSICAL studies on the phage P22 DNA molecule show it to be terminally redundant and circularly permuted (RHOADES, MACHATTIE and THOMAS, in preparation). In these respects it is similar to the DNA of phages T2 and T4 (THOMAS and RUBENSTEIN 1964; THOMAS and MACHATTIE 1964; THOMAS 1966; MACHATTIE, RITCHIE, RICHARDSON and THOMAS 1966). The genetic linkage maps of phages T2 and T4 are circular (Foss and STAHL 1963; STREISINGER, EDGAR and DENHARDT 1964; BAYLOR, HESSLER and BAIRD 1965). The immediate prediction is that the phage P22 linkage map should also be circular (THOMAS 1966).

To test this prediction, a number of temperature sensitive (*ts*) mutants of phage P22 have been isolated and mapped. Mapping of these mutants has greatly increased the length of the map over that previously reported by LEVINE and CURTISS (1961), who mapped only plaque-morphology mutants. We find that a circular arrangement of loci is necessary (and sufficient) to account for all the data.

MATERIALS AND METHODS

Bacteriophage strains: Wild-type phage P22 and the plaque-morphology mutations m_3, c_1, c_2 and h_{21} have been previously described (LEVINE 1957; LEVINE and CURTISS 1961).

The five *ts* mutants used in this report were isolated from wild-type phage P22 mutagenized with 1-methyl-3-nitro-1-nitrosoguanidine. These mutants were selected to produce wild-type plaques at 25° and to form no plaques at 37°. On infection in liquid, each produces 10^{-4}–10^{-5} plaque-forming units (pfu) at 37° as compared to 25°. Strong complementation for phage production is observed in mixed infection, arguing that these mutants belong to distinct functional units. A more complete description of the isolation and characterization of these and other temperature-sensitive mutants will be presented in a later publication.

Extraneous mutations in the *ts* phages were eliminated by a series of crosses to non-temperature-sensitive stocks. The *ts* phages were first crossed to phages carrying *m c h* mutant alleles. Temperature-sensitive progeny of the *m c h* phenotype were then backcrossed to wild-type phage P22, and *ts* phage carrying the wild-type alleles of the plaque-morphology loci were isolated. These were again crossed to the triple mutant particles and appropriately marked *ts* phage were selected.

A note on nomenclature: As *ts* mutants were found, they were assigned isolation numbers. The *ts* mutants were complemented against each other and each complementation group assigned a number: *ts* 1, *ts* 2, etc. Within each complementation group, different mutants are designated

[1] This paper is dedicated to Professor Ralph E. Cleland of the Department of Botany at Indiana University on the occasion of his 75th birthday. The role he played in the graduate training of one of us (M.L.) is fondly acknowledged. It is a pleasure to wish him continued good health and long service to the scientific community.

[2] Investigation supported by grant NIH GM 09252–05 from Public Health Service.

[3] Supported by Training Grant NIH 5T01 GM 00071–10 from Public Health Service.

as *ts* 1.1, *ts* 1.2, etc. Two *ts* loci are identified in the literature by isolation numbers. These are B9 (COHEN and LEVINE 1966) and G5 (ISRAEL, ANDERSON, and LEVINE 1967). These designations are now replaced by *ts* 9.1 and *ts* 5.1 respectively.

Bacterial strains: Wild-type *Salmonella typhimurium* LT2 was used for crosses and preparation of stocks. A galactose requiring mutant was used for plating.

Media: M9-CAA medium (SMITH and LEVINE 1964) was used for all crosses and preparations of stocks. All dilutions were made in buffered saline and all platings were made on indicator agar (BRESCH 1953; LEVINE and CURTISS 1961).

Phage crosses: For crosses between *ts* and plaque-morphology mutant phage, bacteria were grown to 10^8 cells per ml at 37°, then cooled to room temperature for infection. A mixture of two phages at a multiplicity of approximately 10 for each parent was added to one ml of the bacteria. After ten minutes, anti-P22 serum was added (final K = 2.3) to inactivate unadsorbed phage. At 20 minutes the bacteria were diluted 1:4000 in M9-CAA. The infected cells were lysed by shaking with $CHCl_3$ at 180 minutes. Diluted phage suspensions were plated and scored after 12 to 18 hours incubation at 37°. "Ghost plaques," which appeared on some platings of *ts* phage when incubated at 37°, were eliminated by incubation for 30 to 60 minutes at 43° immediately after plating.

The procedure for the *ts* × *ts* crosses was the same, except that suitable dilutions of phage were also plated and incubated at 25°. To determine the recombination frequencies between *ts* loci, the number of pfu per ml at 37° was doubled and divided by the pfu per ml found at 25°. This is necessary since only one recombinant class, the non-temperature-sensitive type, is recovered at 37°.

To minimize variability among the various crosses, all recombination frequencies presented in this paper have been normalized to the standard distances between plaque-morphology markers shown in Fig. 1 (after LEVINE and CURTISS 1961). The normalization factor generally used was the standard m_3-c distance (m_3-c_2 = 18.5; m_3-c_1 = 19.7) divided by the m_3-c distance determined in the cross. In some crosses the results were normalized to the c_1-h_{21} distance (c_1-h_{21} = 6.8). Data for normalization for the crosses in Table 1 are from the platings on indicator agar at 37°. The distances between plaque-morphology markers in the *ts* × *ts* crosses (Table 2) were determined from platings on indicator agar at 25°. The values for normalization factors used in this paper ranged from 0.73–1.35.

Foss and STAHL (1963) described a method for demonstrating map circularity in a single cross. This method was used in one experiment in this report. Bacteria were grown as usual, centrifuged, washed twice in buffered saline, and starved by aeration in buffered saline for 30 minutes at 37°. A mixture of two phages at multiplicities of about 10 for each parent was added to 1 ml of bacteria at 37°. After 5 minutes, anti-P22 serum was added (final K = 1.2) and 10 minutes later the mixture was diluted 1:4000 into 200 ml M9-CAA at room temperature. The time of dilution into M9-CAA is, in effect, the beginning of the infection. Twenty-ml aliquots were removed at 15 minute intervals, chloroformed, diluted and plated at 37°. Platings of the first samples which contained recombinant phage were scored. The premature lysis by chloroform liberated phages which had matured early and in which the parental linkage of markers tended to be preserved. The method for analysis of the data is explained in the RESULTS section.

RESULTS

Mapping of ts *loci by crosses with plaque-morphology markers:* The normalized distances between the five *ts* loci and the various plaque-morphology markers

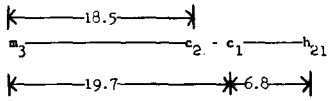

FIGURE 1.—Linkage of four plaque-morphology markers of phage P22 (from LEVINE and CURTISS 1961).

TABLE 1

Results of crosses between temperature-sensitive loci and morphological markers

ts mutant	Cross	Total progeny	Normalized distances		
			ts—*m*	*ts*—*c*	*ts*—*h*
*ts*11.1	*ts*11.1 × m_3c_1	2428	13.9	20.6	. .
	*ts*11.1m_3c_1 × WT	1885	11.0	22.6	. .
*ts*12.1	*ts*12.1 × $m_3c_1h_{21}$	1990	19.8	2.0	5.5
	*ts*12.1$m_3c_1h_{21}$ × WT	3519	19.9	1.7	5.3
*ts*3.1	*ts*3.1 × c_1h_{21}	3001	. . .	15.7	11.9
	*ts*3.1c_1h_{21} × WT	2317	. . .	18.4	12.4
*ts*1.1	*ts*1.1 × $m_3c_2h_{21}$	1484	16.1	20.4	20.0
	*ts*1.1$m_3c_2h_{21}$ × WT	2960	25.2	25.1	24.1
*ts*2.1	*ts*2.1 × $m_3c_2h_{21}$	2610	19.2	21.7	20.0
	*ts*2.1$m_3c_2h_{21}$ × WT	4374	22.9	22.2	21.4

Order of Genes Found in These Crosses

*ts*11.1 m_3 c_2 c_1 *ts*12.1 h_{21} *ts*3.1

are presented in Table 1. Three of the five *ts* loci, *ts* 11.1, *ts* 12.1 and *ts* 3.1 can be mapped in unique locations: *ts* 11.1 to the left of m_3; *ts* 12.1 to the right of c_1, but to the left of h_{21}; and *ts* 3.1 to the right of h_{21}. The linear map at the bottom of Table 1 represents the order of these three *ts* and four plaque-morphology loci.

In contrast *ts* 1.1 and *ts* 2.1 cannot be unequivocally positioned from the results of these crosses. They appear to be about equidistant from each of the plaque-morphology markers.

Mapping of the ts *mutations with respect to each other:* A series of crosses between *ts* mutants was undertaken to determine the map positions of *ts* 1.1 and *ts* 2.1.

Two crosses were carried out between each pair of *ts* mutants. The crosses were reciprocal with respect to the alleles at the *m*, *c*, and *h* loci carried by the parent phages. The results of these crosses are presented in Table 2.

Locus *ts* 1.1 shows considerably closer linkage to *ts* 11.1 (12.9% recombination; cross 1) than to *ts* 12.1 (22.5%; cross 2). Since the distance *ts* 11.1–*ts* 12.1 (18.2%; cross 3) is shorter than *ts* 1.1–*ts* 12.1, *ts* 1.1 cannot be between *ts* 11.1 and *ts* 12.1. The order of these three loci must be *ts* 1.1–*ts* 11.1–*ts* 12.1.

Locus *ts* 2.1 is linked to *ts* 1.1 (9.4% recombination; cross 4). *ts* 2.1 cannot be between *ts* 1.1 and *ts* 11.1 as it is more weakly linked to *ts* 11.1 (15.8%; cross 5) than is *ts* 1.1. Therefore, *ts* 2.1 must be to the left of *ts* 1.1 and the order of the four loci can be written: *ts* 2.1–*ts* 1.1–*ts* 11.1–*ts* 12.1. *ts* 2.1 is linked to *ts* 3.1 (14.3%; cross 6). Since *ts* 1.1 shows 17.0% recombination with *ts* 3.1 (cross 7), *ts* 3.1 cannot be between *ts* 1.1 and *ts* 2.1. The order of these three genes in relation to the other two must then be: *ts* 3.1–*ts* 2.1–*ts* 1.1–*ts* 11.1–*ts* 12.1.

From the crosses to the plaque-morphology markers (Table 1) we know *ts* 3.1 is linked to *ts* 12.1. This is confirmed by the results of cross 8 (14.1% recombination).

TABLE 2

Results of crosses between temperature-sensitive loci

Cross			Normalized *ts*—*ts* distance	Average distance
1	*ts*11.1 $+c_1h_{21}$	× *ts* 1.1m_3 + +	12.2	12.9
	*ts*11.1m_3 + +	× *ts* 1.1 $+c_2h_{21}$	13.6	
2	*ts* 1.1$m_3c_2h_{21}$	× *ts*12.1 + + +	24.2	22.5
	ts 1.1 + + +	× *ts*12.1$m_3c_1h_{21}$	20.8	
3	*ts*11.1$m_3c_1h_{21}$	× *ts*12.1 + + +	18.2	18.2
	*ts*11.1 + + +	× *ts*12.1$m_3c_1h_{21}$	18.2	
4	*ts* 1.1$m_3c_2h_{21}$	× *ts* 2.1 + + +	8.6	9.4
	ts 1.1 + + +	× *ts* 2.1$m_3c_2h_{21}$	10.1	
5	*ts*11.1$m_3c_1h_{21}$	× *ts* 2.1 + + +	14.1	15.8
	*ts*11.1 + + +	× *ts* 2.1$m_3c_2h_{21}$	17.5	
6	*ts* 2.1m_3 $+h_{21}$	× *ts* 3.1 $+c_1$ +	16.4	14.3
	ts 2.1 $+c_2$ +	× *ts* 3.1m_3 $+h_{21}$	12.1	
7	*ts* 1.1$m_3c_2h_{21}$	× *ts* 3.1 + + +	17.5	17.0
	ts 1.1 + + +	× *ts* 3.1$m_3c_1h_{21}$	16.5	
8	*ts* 3.1$m_3c_1h_{21}$	× *ts*12.1 + + +	12.9	14.1
	ts 3.1 + + +	× *ts*12.1$m_3c_1h_{21}$	15.3	
9	*ts*11.1 $+c_1$ +	× *ts* 3.1m_3 $+h_{21}$	21.1	21.3
	*ts*11.1m_3 $+h_{21}$	× *ts* 3.1 $+c_1$ +	21.6	
10	*ts* 2.1$m_3c_2h_{21}$	× *ts*12.1 + + +	23.0	22.2
	ts 2.1 + + +	× *ts*12.1$m_3c_2h_{21}$	21.4	

The five *ts* genes are linked. The spatial relationship of these loci to one another is best represented as points on a circle (Fig. 2).

Evidence for circularity in a single four-factor cross (Foss and STAHL 1963): Premature lysis of cells mixedly infected with wild-type phage P22 and a quadruple mutant, *ts* 2.1 $m_3c_2h_{21}$, was induced by chloroform 90 and 105 minutes after addition of phage. Table 3 lists the number of phages of the various types found on plating at 37°. The 90 minute lysate produced 0.02 mature phage per cell and the 105 minute sample produced 0.06 phage per cell. The two samples were combined for the following analysis. The effect of premature lysis on the frequency

TABLE 3

Frequencies of genotypes from cross of ts*2.1* m_3 c_2 h_{21} × ts+ m+ c+ h+

Genotype	90 minute sample	105 minute sample	Total
ts+ m_3 *c*+ *h*+	92	235	327
ts+ *m*+ c_2 h_{21}	123	332	455
ts+ m_3 c_2 *h*+	46	71	117
ts+ *m*+ *c*+ h_{21}	24	100	124
ts+ m_3 *c*+ h_{21}	3	21	24
ts+ *m*+ c_2 *h*+	61	84	145
ts+ m_3 c_2 h_{21}	109	269	378
ts+ *m*+ *c*+ *h*+	7589	17155	24744

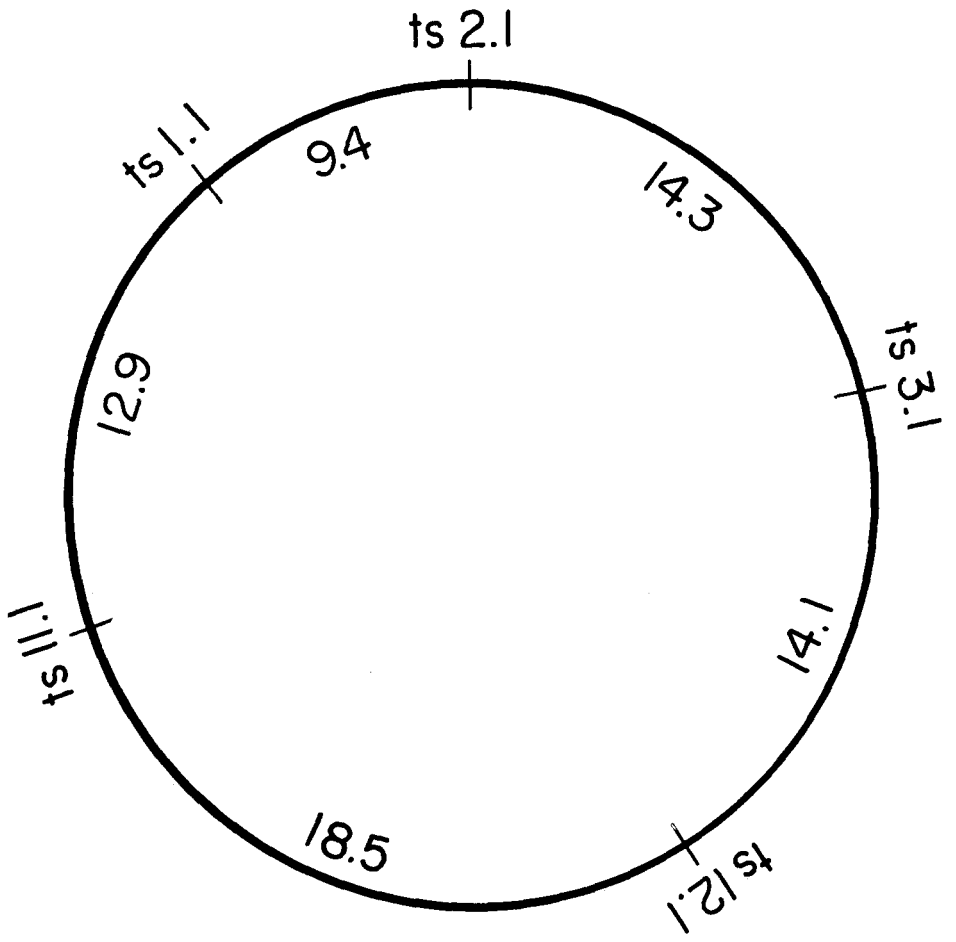

FIGURE 2.—Linkage of five temperature-sensitive genes in phage P22.

of recombination is demonstrated by the finding of only 3.5% recombination between m_3–c_2 as compared to 18.5% in the standard crosses.

The data in Table 3 confirm that *c* and *h* are the most closely linked pair of loci and that *m* is closer to *c* than it is to *h*. The gene order *m*–*c*–*h* is consistent with these data. Since *ts* is farther from *c* than it is from *m* or *h*, *ts* cannot be placed within the *m*–*c*–*h* linkage. The *ts* locus appears to be adjacent to both *m* and *h*, which is consistent with a circular map. For the analysis of these data by the method of Foss and STAHL, the following pairs of loci are adjacent to one another: *ts*–*m*, *m*–*c*, *c*–*h*, *h*–*ts*. The two other pairs, *m*–*h* and *ts*–*c*, do not represent adjacent loci.

Phage particles, recombinant for adjacent markers, were classified according to the presence of one or the other allele of a third locus. If a third locus is unlinked to either marker, it will occur in parental combination with one of the recom-

binant markers as frequently as with the other. If the third locus is linked to the left of the recombinant pair, it will be found in parental combination with the left marker of the pair more often than with the right one. If, however, the third locus is linked more closely to the right marker of the recombinant pair, it will be found in parental recombination with the right marker more often than with the left one.

In Table 4-A through 4-D the linkage of each locus with respect to pairs of adjacent loci is tested and partial maps, derived from each set of data, are presented. The partial maps are circular permutations of each other, and the results confirm a circular map for the 4 loci. Analysis of normal lysates by this method failed to demonstrate circularity. The confirmation of map circularity in a single four-factor cross excludes the possibility that chromosomal alteration in some of

TABLE 4

Linkage of outside markers to recombinant genes in cross of ts*2.1* m_3 c_2 h_{21} × ts^+ m^+ c^+ h^+

A. Method for mapping the markers *h* and *c* with respect to *ts* and *m*.
Number of phage, recombinant for *ts* and *m*, that are

Parental for			Parental for		
c and *ts*	*c* and *m*	P*	*h* and *ts*	*h* and *m*	P
351	495	0.01	444	402	0.15

Conclusions: *c* is to the right of *m*
h is probably to the left of *ts*
The genes are linked in the order *h ts m c*

B. Method for mapping the markers *ts* and *h* with respect to *m* and *c*.
Number of phage, recombinant for *m* and *c*, that are

Parental for			Parental for		
ts and *m*	*ts* and *c*	P	*h* and *m*	*h* and *c*	P
600	351	0.01	169	782	0.01

Conclusions: *ts* is to the left of *m*
h is to the right of *c*
The genes are linked in the order *ts m c h*

C. Method for mapping the markers *ts* and *m* with respect to *c* and *h*.
Number of phage, recombinant for *c* and *h*, that are

Parental for			Parental for		
ts and *c*	*ts* and *h*	P	*m* and *c*	*m* and *h*	P
148	262	0.01	241	169	0.01

Conclusions: *ts* is to the right of *h*
m is to the left of *c*
The genes are linked in the order *m c h ts*

D. Method for mapping the markers *m* and *c* with respect to *h* and *ts*.
Number of phage, recombinant for *h* and *ts*, that are

Parental for			Parental for		
m and *h*	*m* and *ts*	P	*c* and *h*	*c* and *ts*	P
402	579	0.01	833	148	0.01

Conclusions: *c* is to the left of *h*
m is to the right of *ts*
The genes are linked in the order *c h ts m*

* P is the probability that an inequality of the observed magnitude or greater could result from chance.

the individual stocks used in the two factor crosses could have accounted for the deduced gene order.

The map of phage P22: Figure 3 represents the linkage map of phage P22. The map is about 90 recombination units in length when normalized to the m_3–c_2 distance of 18.5 and is approximately three times the length (34 units) of the map found for plaque-morphology markers reported previously (LEVINE and CURTISS 1961).

DISCUSSION

The genetic data presented in this report show that the vegetative linkage map of the temperate phage P22 can be represented as a single circle. This is consistent with the evidence that the phage P22 DNA molecule is circularly permuted and terminally redundant (RHOADES, *et al.*, in preparation).

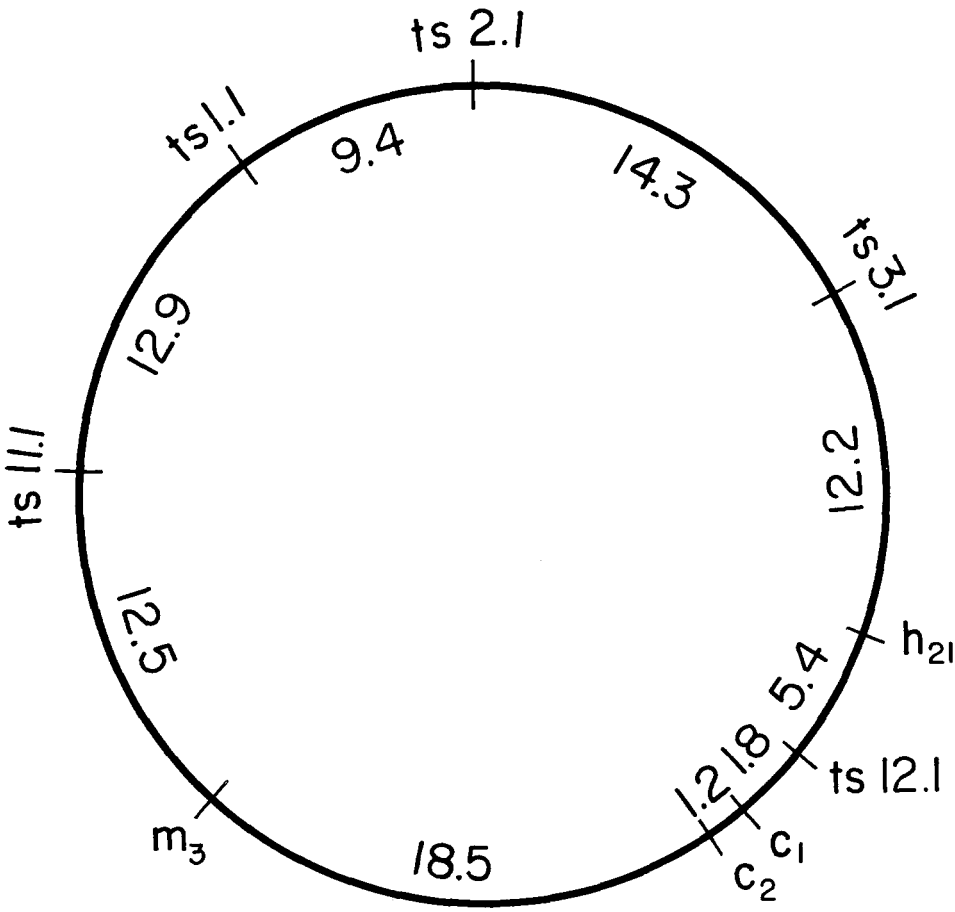

FIGURE 3.—The linkage map of phage P22.

Bacillus temperate phage PK also has a circular map (RUTBERG, SANDSTROM, and NILSSON 1967).

With regard to chromosome structure, phage P22 resembles the virulent T-even phages more than the temperate phage λ. Phages T4 and T2 also have circular linkage maps and circularly permuted and terminally redundant DNA molecules (THOMAS and RUBENSTEIN 1964; THOMAS and MACHATTIE 1964; THOMAS 1966; MACHATTIE, RITCHIE, RICHARDSON and THOMAS 1966). In contrast, the well-mapped phage λ has a linear vegetative linkage map (CAMPBELL 1961) and its DNA molecule is not circularly permuted (HOGNESS and SIMMONS 1964). This should not, of course, mask the obvious genetic similarities of the two temperate phages (LEVINE and CURTISS 1961; KAISER 1957; CAMPBELL 1961). Most striking is the apparent analogous linear arrangement of loci in the two prophages (CALEF and LICCIARDELLO 1960; ROTHMAN 1965; SMITH and LEVINE 1965; SMITH, in press). However, the prophage linkage map for phage P22 is a linear derivative of a circular vegetative map, while that for λ appears to be a specific circular permutation of a linear vegetative map.

SUMMARY

A series of *ts* mutants was isolated in phage P22. When these were crossed with plaque-morphology markers and among themselves, the map of P22 was found to be about three times longer (approximately 90 units) than previously reported. A circular arrangement for the markers is required to account for the data.

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