MAPPING OF TEMPERATURE-SENSITIVE MUTANTS IN BACTERIOPHAGE T5[1]

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T5, one of the classical T phages of *Escherichia coli*, has been the subject of many studies, but its genetic analysis has been sporadic. Beginning with heat-stable (*st*) mutants (ADAMS and LARK 1950; ADAMS 1953), there have been reports of mutants with altered host range (LANNI and LANNI 1956), serological behavior (LANNI and LANNI 1957), lysis-inhibiting properties (Y. T. LANNI 1958), and buoyant density and sedimentation rate (HERTEL, MARCHI and MÜLLER 1962; LARK 1962). Host-range and serological differences are correlated, as are differences in heat-stability and density. Several of the aforementioned mutants show altered plaque type. Additional plaque-type mutants of various kinds are easily isolated from routine assay plates, especially with *E. coli* strain F as the host.

Genetic recombination in T5 was first reported by ADAMS (1951, 1952) in crosses between T5 and the taxonomically related phages PB. BG3, and 29 alpha, which were independently isolated from natural sources. T5, PB, and their hybrids have been used in studies of the cytological effects of phage infection (MURRAY and WHITFIELD 1953) and the genetic control of host-range and serological specificity (FODOR and ADAMS 1955; FODOR 1957; WASSERMANN 1959; see also F. LANNI 1958). The considerable mutual exclusion in mixed infection and the multiple hereditary differences between the parents limit the value of these interstrain crosses for genetic mapping. More recently, HERTEL *et al.* (1962) reported recombination between plaque-type and density markers in T5 mutants, and M. NESSON (personal communication from R. S. EDGAR) observed recombination between temperature-sensitive (*ts*) mutants of the kind to be described here. (*Note:* The *st* phenotype involves an increased stability of the free phage particle in media of low ionic strength, whereas the *ts* phenotype involves a decreased ability to grow at a moderately elevated temperature).

A suitably comprehensive genetic map is needed in conjunction with studies of T5 progressing in various laboratories and in comparative bacterial virology. For example, under certain well defined conditions the T5 DNA molecule can be sectioned artificially during transfer from the infecting phage particle to the host cell. The transferred section amounts to about 8 percent of the whole DNA

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molecule (McCORQUODALE and LANNI 1964; LANNI, McCORQUODALE and WILSON 1964) and appears to perform some but not all the functions of the whole molecule (LANNI and McCORQUODALE 1963). Suitably distributed genetic markers would be very useful in continuing these studies.

As a second and related example, the DNA molecules from a population of phage T2 appear to be cyclically permuted with respect to nucleotide sequence, whereas T5 DNA molecules appear sequentially homogeneous (THOMAS and RUBENSTEIN 1964). In agreement with the data for T2, the genetic map of the closely related phage T4 is circular (STREISINGER, EDGAR and DENHARDT 1964, confirmed by Foss and STAHL 1963; EDGAR, DENHARDT and EPSTEIN 1964; EDGAR and LIELAUSIS 1964). A prediction to be tested is that the T5 map is noncircular.

Experience with phages λ (CAMPBELL 1961) and T4 (EDGAR and LIELAUSIS 1964) indicates that *ts* mutations scatter widely over the genome. We therefore chose *ts* mutants for this initial study of the gross structure of the T5 genome. Our mutants grow in *E. coli* strain F at 30°C but not (or very little) at 43°C. A preliminary report has appeared (FATTIG and LANNI 1964).

MATERIALS AND METHODS

Bacteria: E. coli strain F (Y. T. LANNI 1958), a fast-adsorbing host for T5, was used in all the experiments reported here.

Wild-type phage: The T5 wild-type strain was originally obtained from DR. S. E. LURIA and has been used for many years in this laboratory. Some growth characteristics are presented in Table 1.

Media and plating procedures: The nutrient agar media and broth are described by Y. T. LANNI (1958). The synthetic MGM medium and buffer are those of LANNI (1961). Calcium (10^{-3} M) was present in all media except the broth and MGM used for growing bacteria.

Plates for 30°C or 37°C incubation contained about 35 ml of bottom agar. Those incubated at 43°C contained about 45 ml. For some uses, plates poured with bottom agar were predried at 30°C for about 2 hours with the lid half removed. One drop of undiluted bacteria from an overnight broth culture was used for 30°C agar-layer platings, and two drops for 43°C platings. About 0.7 ml of a 1:10 dilution of an overnight culture was used for direct seeding of predried plates.

Induction and isolation of mutants: Mutants were isolated from wild-type stocks grown in the presence of 5-bromodeoxyuridine (BUDR). With the exception of *ts*1, *ts*2, and four BUDR-induced mutants isolated by M. NESSON and kindly supplied by DR. R. S. EDGAR, mutants were induced by a modification of the technique of FERMI and STENT (1962). Bacteria were grown in MGM medium to about 7.5×10^7 cells/ml, washed once in buffer, and concentrated to about 1.5×10^8 cells/ml. The cells were starved, without aeration, for 45 min at 37°C in buffer, BUDR

TABLE 1

*Some growth characteristics of wild-type T5**

	30°C	37°C	43°C
Minimal latent period	49 min	37 min	31 min
Maximal latent period (approximate)	90 min	60 min	48 min
Average burst size	750	500	300
Relative efficiency of plating	1.00	1.00	0.94

* Growth parameters from one-step growth curves with low multiplicity of infection (0.06–0.13). The relative plating efficiency was measured for free phage (not preadsorbed).

was added to a final concentration of 250 μg/ml, and the cells were infected with wild-type phage at a multiplicity of infection (m.o.i.) of about 4. After 15 min for adsorption, a mixture of glucose and NH_4Cl was added to convert the buffer to MGM, and the culture was gently aerated. After an additional 30 min the phage-bacterium complexes were diluted 2500-fold into MGM containing 250 μg/ml of thymidine. This dilution was subdivided into a number of small tubes, and after 120 min at 30°C one drop of chloroform was added to each tube. Ordinarily only one mutant was isolated from each small tube to insure that the mutants would be derived from independent mutational events.

Mutants *ts*1 and *ts*2 were obtained essentially as described above, except that the lysate was made by adding BUDR (100 μg/ml) to a broth culture of bacteria (2×10^7 cells/ml).

The procedure for isolating mutants is based on that of EDGAR and LIELAUSIS (1964). Mutagenized phage was pre-adsorbed to bacteria and plated by the agar-layer method on predried plates. The plates were incubated 5 hr at 30°C, then transferred to a 41°C incubator overnight. Small plaques, which were presumed to have resulted from arrested growth of *ts* mutants, were picked with sterile pins and transferred to the surface of two predried agar plates which had been seeded with bacteria. One plate was incubated at 41°C, the other at 30°C. Plaque picks which produced a clearing only on the 30°C plate were further tested by picking from this plate, diluting, and plating in agar-layer at 30°C and at 43°C. Lysates of those isolates which gave no plaques at 43°C were made by stabbing a single plaque from the 30°C plate and rinsing into bacteria (5×10^7 cells/ml) in broth at 30°C. After clearing (usually 5 to 7 hrs) the lysates were centrifuged at 2500 rev/min, chloroform (0.5 drop/ml) was added to the supernatants, and the stocks were stored in a refrigerator. About one percent of the progeny in a mutagenized lysate were *ts* mutants.

Revertant frequencies in stocks were measured by plating at 43°C (revertants) and 30°C (total phage).

Before use, a dilution of each phage stock was warmed for 15 min at 45°C to dissociate phage clumps (LANNI 1959).

Mutants obtained from EDGAR have been given the suffix E.

Complementation tests: Spot tests. Overlapping spots of two mutants (at about 5×10^7 phage/ml) were made with a standard loop on predried plates seeded with bacteria. Control spots, in which the parental strains were overlapped by themselves, were placed on the same plate as the mixed spot test. The plates were placed in a 43°C incubator and left overnight. Clearing in the overlap region, where bacteria were mixedly infected, was scored as complementation. Failure of the overlap region to become cleared was scored as noncomplementation.

Liquid tests. Complementation relationships were also measured by comparing the average burst size of mixedly infected bacteria at 30°C and 43°C in broth. Bacteria at 10^8 cells/ml were infected at 0°C with a 1:1 mixture of two mutants (total multiplicity of infection about 10). After 60 min, when adsorption was largely complete in those cases where it was measured, dilutions were made into 30°C and 43°C growth tubes. Chloroform was added at 60 min to the 43°C tube and at 120 min to the 30°C tube. Dilutions of the lysates were plated and incubated at 30°C, and the average phage yield per input bacterium was calculated. Control lysates of each mutant (m.o.i. about 10) at the two temperatures were prepared and assayed in the same fashion.

The 43°C control lysates give a measure of the "leakiness" of the individual mutants.

Recombination tests: Two-factor crosses were made by infecting bacteria as described above for liquid complementation tests. The 30°C lysates were assayed at 30°C and 43°C. Since the parental mutant types, and presumably the double-mutant recombinant type, do not form plaques at 43°C, only the wild-type recombinants are scored at this temperature. All progeny phages, however, presumably form plaques at 30°C. The recombination frequency is expressed as twice the plaque count at 43°C divided by the plaque count at 30°C. The recombination percentage is 100 times the recombination frequency.

A few crosses, in which the m.o.i. was less than 6 or more than 14, were excluded in this analysis, as were a few crosses in which the ratio of minority to majority parent was less than 0.67 (DOERMANN and HILL 1953).

The data presented are based on 150 total crosses. In repeated crosses (pooling data for mem-

bers of the same cistron) the average deviation ranged from 0.4 to 30 percent of the mean. For the whole set of crosses the average value of the average deviation was 7.8 percent.

Experiments were performed to determine what proportion of phage-yielding bacteria were mixed yielders. Two mutants (*ts*34 and *ts*37), chosen for their high recombination frequency, were crossed and the infected complexes were plated at both 30° and 43°C before lysis. About 95 percent of the phage-yielding complexes produced recombinants.

Experiments were also done to estimate the amount of "lysis from without" at the multiplicity of infection used in crosses. At the usual m.o.i. of about 10, the loss of infected bacteria is less than 10 percent. At much higher m.o.i. (20–40) the loss is about 25 percent.

RESULTS

Characterization of mutants: About one third of the *ts* mutants gave plaques resembling wild-type plaques in size. The remainder gave somewhat smaller plaques whether plated as free phage or after adsorption to bacteria.

Where revertants were encountered, their frequency in the stocks used in crosses and complementation tests ranged from 2×10^{-3} to 2×10^{-7}, with most values falling near 10^{-5}. With some mutants, no revertants were encountered in the routine platings made to date, but no special effort was made to measure exceptionally low frequencies.

Under the conditions of the crosses (30°C, m.o.i. about 10) the phage yield for individually tested mutants ranged from about 50 to about 400 particles per input bacterium, most values being greater than 100 (compared with about 340 for the wild type under the same conditions). The yield in crosses generally was greater than for either mutant alone and occasionally exceeded the sum of the individual values, suggesting complementation even at 30°C. The yields for selected mutants at 30°C and 43°C are given later.

Of the two reported mutants (*ts*5 and *ts*6E) giving a 30°C yield of about 50, one (*ts*5) also produces small plaques of variable size and is not desirable for mapping; the available data for this mutant are reported here, but it is no longer being used in crosses. A few mutants appear to carry a small-plaque marker which segregates out among the ts^+ recombinants in crosses.

Complementation tests: The 25 mapped mutants (listed in Figure 1 and Table 3) were spot-tested for complementation in all pairwise combinations. All the tests were positive except those between mutants within each of the following groups: 11 and 41; 3, 9, 18, 39, and 44; 45 and 6E; 6, 20, 7E, and 8E; 4, 36, and 43. Some of the results are given in Table 2. The spot tests are not completely unambiguous in some instances, as the results with mutants 6, 20, 7E, and 8E indicate. However, the tests are generally satisfactory for assigning mutants to new or previously identified cistrons, and they have shown *ts*40 to be a double mutant having lesions in each of two known cistrons. The interpretation of spot-test results depends upon clearing or nonclearing of bacteria on a plate, and the test is reliable only when the mutant controls do not clear. Because the results obtained are influenced by variability in incubator temperature and by the character of the individual mutants themselves, the tests in liquid, where the temperature can be more precisely controlled, may be a better measure of complementation relationships.

TABLE 2

Results of crosses and complementation tests with selected mutants

Mutant or cross	Percent recombination*	Spot test	Average burst 30°C	Average burst 43°C	Ratio 30°C/43°C	43°C burst as percent of wild
Wild	. . .	.	340**	68.0**	5.0	100
2	. . .	.	97	0.408	240	0.60
3	. . .	.	414	0.195	2100	0.29
4	. . .	.	374	2.59	140	3.8
6	. . .	.	199	0.366	540	0.54
18	. . .	.	165	0.176	940	0.26
20	. . .	.	221	0.740	300	1.1
36	. . .	.	124	0.746	170	1.1
37	. . .	.	104	0.786	130	1.2
39	. . .	.	272	0.482	560	0.71
43	. . .	.	72	0.326	220	0.48
45	. . .	.	73	0.189	390	0.28
7E	. . .	.	215	2.34	92	3.4
8E	. . .	.	439	0.115	3800	0.17
3 × 18	0.01[1]	0	343	0.546	630	0.80
3 × 39	0.005[1]	0	378	0.518	730	0.76
18 × 39	0.01[1]	0	406	0.263	1500	0.39
45 × 6E	0.89[1]	0	372	0.330	1100	0.49
4 × 36	0.87[2]	0	239	1.85	130	2.7
4 × 43	0.98[1]	0	270	0.527	510	0.77
36 × 43	1.95[2]	0	242	0.970	250	1.4
6 × 8E	0.63[1]	0	343	2.09	160	3.1
6 × 20	1.10[2]	?	270	1.33	200	2.0
6 × 7E	1.51[2]	+	273	3.09	88	4.5
8E × 20	0.73[1]	?	296	0.364	810	0.54
8E × 7E	1.49[1]	?	280	1.86	150	2.7
20 × 7E	0.80[2]	?	203	1.13	180	1.7
2 × 40	0.01[1]	0	103	0.026	4000	0.04
37 × 40	0.00[1]	0	104	1.13	92	1.7
39 × 45†	1.58[2]	+	396	54.3	7.3	80
36 × 7E†	4.8[1]	+	470	41.7	11	61
4 × 7E†	6.1[2]	+	346	23.1	15	34
2 × 37†	23[1]	+	393	48.0	8.2	71

* Not corrected for revertants; corrections would be small. A superscript denotes the number of crosses on which the average value shown is based. Mutants *ts*11 and *ts*41 gave 0.04 percent recombination and a negative spot test, but were not tested quantitatively for complementation.

† Intercistronic crosses. All others are intracistronic, with *ts*40 being judged to have lesions in two cistrons.

** The lower average burst sizes reported here for wild-type phage (compare Table 1) are associated with the higher m.o.i. (10 instead of single infection). We have no explanation.

Table 2 shows that the average burst of wild-type phage is reduced 5-fold at 43°C from the value at 30°C. The reduction for *ts* mutants, however, is more striking, ranging from about 100-fold to over 1000-fold. For each mutant studied, this reduction is an inverse measure of its "leakiness," where leakiness is defined as a partial expression of wild-type phenotype. The average burst at 43°C from mixed infections, expressed as percentage of the wild-type 43°C value, permits the separation of the data into two groups. In one group the 43°C yield is about

35 to 80 percent of the wild-type yield, indicating that wild-type functions have been considerably restored in the mixedly infected complexes. In such instances (*ts*2 × *ts*37, *ts*45 × *ts*39, *ts*4 × *ts*7E, *ts*36 × *ts*7E) the mutants are judged to reside in different cistrons. In the other group, the yield is 0.04 to 4.5 percent of the wild-type yield. Here it appears that very little complementation occurs, and the mutants have been assigned to the same cistron. The 43°C yields for crosses in Table 2 have not been corrected for the slight leakiness of the parental strains at this temperature. A correction, however, would decrease the calculated yields for noncomplementing pairs to a proportionally greater extent than for complementing pairs and would accentuate the differences between the two groups. The negligible intracistronic complementation observed so far in T5 contrasts with the situation in T4 (EDGAR, DENHARDT and EPSTEIN 1964).

The results indicate that the 25 mutants used in mapping experiments fall into 14 cistrons. Mutants 3, 9, and 44 were isolated from the same mutagenized stock, and their similar 30°C yields, revertant frequencies, and plaque morphologies suggest that they resulted from the same mutational event. Two additional mutants, *ts*7 and *ts*32, appear to represent two new cistrons, but they have characteristics which make them undesirable in mapping and have been omitted from any general considerations in this report.

Mapping experiments: A map of the T5 genome (Figure 1) has been constructed from the data of two-factor crosses (Table 3). Because of negative interference, certain order assignments remain tentative or undecided (see DISCUSSION). The total map, summed from smallest adjacent intervals, comprises about 110 recombination units. Cistrons marked with more than one mutant appear to be clustered in the central region of the map. However, one of the two lesions in *ts*40 occurs in the *ts*37 cistron, and a mutant recently isolated by DR. LING CHU in this laboratory seems to fall in the *ts*33 cistron. The data from intracistronic crosses are summarized in Table 2 and Figure 1.

The fact that double-mutant recombinants do occur in the progeny of a cross has been established by complementation spot-testing of random plaque picks from 30°C platings of the progeny of several intercistronic crosses. In every instance, plaques which failed to complement either parent have been found.

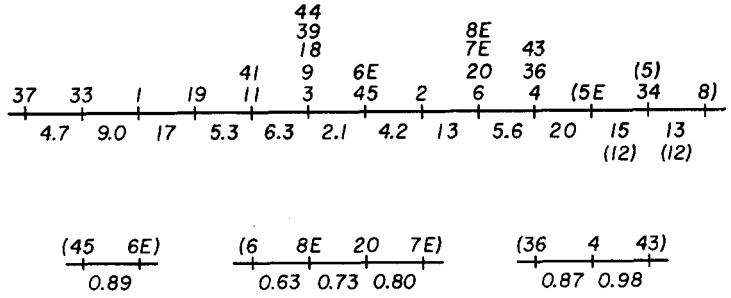

FIGURE 1.—Linkage map of *ts* mutants of phage T5, showing recombination percentages between adjacent identified cistrons (above) and within cistrons (below). Values in parentheses are for *ts*5, which has not been ordered relative to *ts*34. Parentheses around a group of mutants indicate that the orientation of the corresponding segment in the main linkage map is uncertain.

TABLE 3

Recombination percentages for intercistronic crosses between* ts *mutants*

	ts mutant													
ts mutant	37	33	1	19	41 11	44 39 18 9 3	6E 45	2	8E 7E 20 6	43 36 4	5E	5	34	8
37	..	4.7^{3}	12^{2}	24^{1}	24^{2}	..	21^{1}	23^{1}	29^{2}	34^{2}	38^{1}	..	34^{6}	36^{1}
33	..	..	9.0^{1}	16^{1}	22^{1}	..	18^{1}	..	..	..	..	..	30^{1}	..
1	..	..	..	17^{1}	18^{2}	18^{2}	17^{1}	19^{1}	..	29^{1}	..	40^{1}	31^{1}	37^{2}
19	..	..	..	..	5.3^{2}	9.6^{1}	9.0^{2}	14^{1}	20^{3}	..	..	..	29^{2}	..
41.11	..	..	..	..	..	6.3^{1}	6.6^{1}	..	18^{1}	17^{1}	..	..	31^{1}	..
44,39,18,9,3	..	..	..	..	..	..	2.1^{3}	5.0^{2}	13^{3}	16^{4}	..	30^{2}	31^{1}	32^{2}
6E,45	..	..	..	..	..	..	..	4.2^{1}	12^{3}	15^{2}	24^{1}	..	27^{3}	27^{1}
2	..	..	..	..	..	..	..	..	13^{3}	16^{2}	..	30^{1}		
8E.7E,20,6	..	..	..	..	..	..	..	..	..	5.6^{6}	22^{2}	..	24^{3}	20^{2}
43,36,4	..	..	..	..	..	..	..	..	..	..	20^{4}	22^{3}	20^{5}	21^{5}
5E	..	..	..	..	..	..	..	..	..	..	..	12^{1}	15^{3}	18^{4}
5	..	..	..	..	..	..	..	..	..	..	..	..	..	12^{4}
34	..	..	..	..	..	..	..	..	..	..	..	..	..	13^{3}
8	..	..	..	..	..	..	..	..	..	..	..	..	..	..

* A superscript denotes the number of crosses on which the average value shown is based. Data for mutants in the same cistron are pooled.

DISCUSSION

Since the longest gap in the present map of T5 (Figure 1) measures about 20 recombination units, well below the maximal value of about 35 percent recombination in two-factor crosses, the results indicate that the T5 genome is a single linkage structure. This conclusion agrees with the fact that T5 DNA can be extracted as a single molecule (HERSHEY, BURGI and INGRAHAM 1962; THOMAS and PINKERTON 1962; and others).

Our results also indicate that the genetic map of phage T5 is noncircular. This conclusion is based on the observation that mutants which are judged to be progressively farther apart along the map constructed from the smaller recombination values give progressively greater recombination values (up to the maximum of about 35 percent) when crossed directly.

If the map actually were circular, our results would imply that the present map covers not more than about 50 percent of the genome. The general theory of temperature-sensitive mutants (see EDGAR and LIELAUSIS 1964) implies that such mutants can be produced in many regions of the genome, but some regions may be missed. Although the procedures used in obtaining T5 mutants may select against some regions (e.g., those involved in early intracellular functions), it seems unlikely that enough of a circular map would be missed to invalidate the conclusion of noncircularity. In T4, *ts* mutants sufficed by themselves to establish map circularity (EDGAR and LIELAUSIS 1964), even though a gap of 30 recombination units exists in the *ts* map. In the present work, the placement of two of the first five mutants isolated (*ts*1 and *ts*5) effectively defined the end regions of the present map, and the mapping of 20 more mutants extended each end by only

about 12 recombination units. We therefore do not expect that mapping additional mutants will reveal a new genetic segment comparable in size to that already mapped.

The noncircularity of the T5 map agrees with evidence (THOMAS and RUBENSTEIN 1964) that T5 DNA molecules are not cyclically permuted with respect to nucleotide sequence, in contrast to the situation with the T4-related phage T2.

It is of interest to compare the respective DNA sizes and map lengths in T5 and T-even phages. The molecular weight of T5 DNA, estimated as about 80×10^6, is about two-thirds that of T-even DNA (see, for example, BURGI and HERSHEY 1963). The length of the T5 map (Figure 1), summed from the smallest adjacent intervals, is about 110 recombination units. The length of the T4 *ts* map (EDGAR and LIELAUSIS 1964), where the elementary intervals are more or less comparable in size to those in the T5 map, is about 500 units, or about 4 to 5 times as great. Subject to further study, the disparity in the two comparisons does not seem very great, contrary to a suggestion from cytological observations (KELLENBERGER 1961). Suitable mapping functions might be helpful in these comparisons, but there is reason to doubt, as indicated later, that our data for T5 can be accommodated by *any* mapping function, as usually conceived.

The intracistronic crosses (Table 2) made it possible to order the mutants within two cistrons (Figure 1). In all cases where two mutants were assigned to the same cistron by complementation tests, the mutants gave very low recombination values. This correlation of functional allelism with close linkage further validates the criteria used in assigning mutants to cistrons. Close linkage is not sufficient in itself, however, to produce a negative complementation test. Thus, *ts*39 and *ts*45, which give only 1.6 percent recombination, are judged to lie in different cistrons because they complement each other in spot tests and, in mixed infection at 43°C, give a yield which is 80 percent that of the wild type. In contrast, *ts*36 and *ts*43, which give 2.0 percent recombination, give a negative spot test and a 43°C yield which is only 1.4 percent that of the wild type.

The non-zero intracistronic map distances permit a preliminary minimal estimate of 1 to 2 recombination units for the average length of T5 cistrons. If cistrons are contiguous (without intervening nongenetic regions), the data therefore suggest that the whole genome may comprise less than 50–100 cistrons. This number may be compared with the 50 or so cistrons (genes) already identified in phage T4 (EDGAR, DENHARDT and EPSTEIN 1964).

We turn finally to some curious mapping difficulties of a type reported long ago for T2 by HERSHEY and ROTMAN (1948, 1949) and apparently still not adequately explained. For convenience, we assign the 14 mapped cistrons to four groups, representing the map segments 37-1, 19-2, 6-4, and 5E-8. The data of Table 3 leave little question that the map order of the four segments is that indicated in Figure 1. Likewise, the ordering of cistrons within a given segment presented no unusual problems, although additional measurements may be desirable in some instances.

The difficulty comes in deciding the orientation of certain segments in the whole map and was first noticed with the 5E-8 segment. Table 3 shows that the

considerable 5E-8 distance makes no significant net contribution in crosses with mutants in the *ts*4 cistron, the nearest available cistron outside the segment. Within experimental error, the same result is observed in crosses with other mutants. In general, the farther a mutant lies to the left of the *ts*4 cistron, the higher the average recombination value, confirming the linkage of the 5E-8 segment to the rest. The data thus give no basis for deciding which mutant, 5E or 8, is at the right end of the map.

The 19-2 segment gave analogous difficulty, but the crosses with the *ts*6 cistron suggest the orientation shown in Figure 1. Crosses between 19-2 and 37-1 mutants were not helpful in this decision.

If the T5 map is linear (unbranched, noncircular), and if we are not merely dealing with a very orderly artifact, the mapping anomalies are a form of negative interference (see HERSHEY 1958). Naming them does not, of course, explain them. We suspect that an explanation different from those given to other forms of negative interference may be needed.

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SUMMARY

Twenty-five BUDR-induced temperature-sensitive mutants of bacteriophage T5 fall into 14 cistrons comprising a single linkage structure. The genetic map for these mutants is linear, not circular, and is about 110 recombination units long. A preliminary estimate suggests that the total number of cistrons does not exceed 50–100. Some mapping anomalies are discussed.

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