A COMPARATIVE GENETIC STUDY OF CONDITIONAL LETHAL MUTATIONS OF BACTERIOPHAGE T4D[1]

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TWO types of conditional lethal mutations have been described for bacteriophage T4D. Amber (*am*) mutants can propagate in *Escherichia coli* K-12 (strain CR63) but are unable to complete a successful infective cycle of growth in strain B (EPSTEIN, STEINBERG and BOLLE 1964). Temperature-sensitive (*ts*) mutants can grow in either strain at 25°C, but unlike the wild-type phage from which they are derived, they are unable to complete a successful growth cycle at higher temperatures (37°C to 42°C) (EDGAR and LIELAUSIS 1964). Thus both mutant classes may be defined as conditional lethals since permissive as well as restrictive growth conditions exist. Mutations of both types have been shown to be distributed widely over the genome of T4D. They also affect a variety of different functions of the phage (EPSTEIN, BOLLE, STEINBERG, KELLENBERGER, BOY DE LA TOUR, CHEVALLEY, EDGAR, SUSMAN, DENHARDT and LIELAUSIS 1963). It would thus appear that the diagnostic phenotypes of these mutations (temperature sensitive for *ts* mutations and inability to grow in B for *am* mutations) are not mutant properties associated with particular gene functions, but rather are properties of specific mutations which occur in many, if not all, of the genes of the organism. In support of this notion, we will show that many *am* and *ts* mutations occur in the same genes.

A gene may be defined as a segment of a genome which contains the hereditary information determining the primary structure of a polypeptide chain. In the absence of information concerning mutational alterations of specific proteins, the most satisfactory operational definition of a gene is derived from complementation tests. Cells containing two functioning genomes with different recessive mutations in the *trans* configuration are expected to exhibit a mutant phenotype if the mutations are in the same gene, a wild-type phenotype if the mutations are in different genes. In practice this test is complicated, but not vitiated, by phenomena of intragenic complementation (see, for example, CATCHESIDE and OVERTON 1958) and gene interaction (LEWIS 1963).

For conditional lethal mutations in phage, complementation interactions may

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be determined from the ability of cells, mixedly infected under restrictive conditions with two different mutant types, to produce progeny virus. Such tests were first employed for the *rII* mutations of T4 (BENZER 1957). Such complementation studies have shown that the amber mutations, with few exceptions, can unambiguously be assigned to cistrons (or genes) since *am* mutations in the same gene do not complement with each other but do complement with *am* mutations in all other genes (EPSTEIN *et al.* 1964). We will show that intragenic complementation between *ts* mutations occurs in many genes, and that the results of complementation tests involving both *ts* and *am* mutants exhibit the same relationships found for a number of genes in bacteria and fungi.

In addition to the results of the complementation studies, a map of phage T4D, combining *am*, *ts* and previously studied mutations will be presented.

MATERIALS AND METHODS

Phage strains, bacterial strains, and most materials and methods have been previously described by EDGAR and LIELAUSIS (1964) and EPSTEIN *et al.* (1964). The source of some phage strains, not described in the papers above mentioned is indicated by reference in the legend to Figure 3.

Complementation tests: Spot tests. Spot-test plates were incubated at high temperature to prevent growth of *ts* mutants. In areas of a plate where drops of two *ts* mutants are overlaid, many bacteria become mixedly infected with the two mutant types. If the two mutants tested complement one another in growth, a full burst of progeny, including many recombinants, is produced. This results in clearing of the spotted area, probably due to multiplication of recombinants. If the two mutants do not complement one another in growth, no progeny will be produced, leaving the spotted area turbid.

The tests were performed as follows. Three ml of top layer agar containing plating bacteria (10 ml S/6 to 100 ml top layer agar) were put in 60 mm petri dishes. After the agar hardened, one drop of each of the mutant phage to be tested (at a concentration of about 5×10^7 phage/ml) was added. The dishes were rocked, to allow the drops to spread, and immediately incubated at 42°C. In each experimental set, appropriate controls of each mutant alone were made. Complete clearing of overlapping spots was classed as complementation. No clearing whatever, within the overlap areas, was taken to indicate no complementation. In some tests, isolated plaques were found within the spotted areas. Provided fewer plaques were found in the control spots, this result indicates that the two mutations tested are within the same functional group but at different sites and that recombinant plaques arose due to "leakage." Such results were also classed as noncomplementation.

Burst size tests: E. coli B bacteria grown to a concentration of about 4×10^7 cells/ml were concentrated by centrifugation and resuspended to give a concentration of 4×10^8 cells/ml. KCN was added to produce a final concentration of 0.004 M. Cells were infected with a multiplicity of four of each phage type. After 5 min, at which time over 90 percent of the phage have adsorbed, the mixture was treated with phage antiserum inactivating more than 99.9 percent of unadsorbed phage. The infected cells were then diluted 10^5-fold and one sample was incubated at 25°C for determinations of recombinant frequency while another sample was incubated at 39.5°C for the complementation tests. To both samples, chloroform was added at 60 min (30 min in some cases, as described under RESULTS) and the lysates obtained were assayed for total viable phage yield (determined from plaque counts of plates incubated at 25°C). Recombinant frequencies were determined as described by EDGAR and LIELAUSIS (1964). In all cases, parallel control experiments were performed consisting of measurements of the burst sizes (phage yield/infected bacterium) of each of the mutants alone and of the wild type at both the high and low temperatures.

RESULTS

Complementation tests. ts × ts: Prior to mapping *ts* mutations (EDGAR and LIELAUSIS 1964), complementation tests were performed in an attempt to group together mutants having common functional defects. Mutations giving no complementation should be located in the same gene and be closely linked. Thus initial mapping experiments could be performed with representatives from each functional group or gene as determined from the complementation tests.

The complementation tests were first performed by the spot test method described in MATERIALS and METHODS. Not all possible pairwise tests among all mutants were performed. When a pair was found which did not complement, one member of the pair was set aside and one mutant was used as a "tester phage" for that functional group. Finally, all members of the same functional group (defined by the tester) were spot tested against one another. These tests revealed a number of different functional groups, some containing many mutants. However, the quantitative complementation tests described below showed in many cases that mutants in different complementation groups as defined by the spot tests nevertheless gave poor complementation when tested quantitatively. Comparisons of the results of the spot tests with the quantitative tests showed that the spot test method is reliable *only* for cases where a negative result (no complementation) is obtained. Nevertheless, the spot tests proved of great value as a first screening test.

Measurement of the burst size from bacteria infected with two mutant types under conditions where either mutant alone gives a very low burst size is a quantitative and thus more sensitive test of complementation than the spot test. The yield of viable phage from bacteria infected with *ts* mutants and incubated at the high temperature (39.5° to 40.5°C) is very low, in most cases less than one phage per infected cell. At these temperatures the yield from wild-type-infected cells is usually 100 to 300 phage per cell. Thus an index of complementation can be taken as the burst size at high temperature from cells infected with two different *ts* mutants as compared to the burst size of wild-type infected complexes.

In addition to the complementation tests, in most experiments the recombination frequency was measured in progeny from complexes permitted to produce phage at 25°C.

We selected three regions of the genome for intensive complementation studies. These regions included all cases where the results of the complementation spot tests were not clear.

The B25–N16 region: The mutants obtained from the random isolation series (series A, N and B, see EDGAR and LIELAUSIS 1964) which were located in the region of the map bounded by the markers B25 and N16 were tested for complementation and recombination in many combinations. This set of mutants is a collection of mutants of independent occurrence, unselected except for their location in this region of the map.

The results of the mapping experiments and complementation tests with these mutants are summarized in Figure 1. The 66 mutations studied are distributed

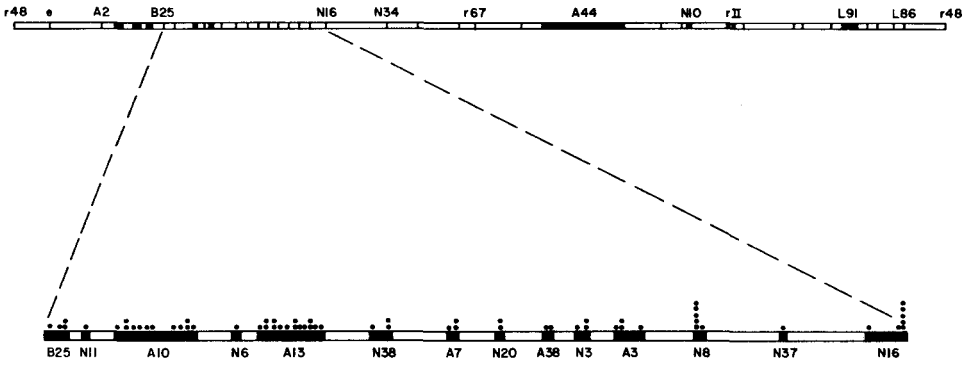

FIGURE 1.—A map of the B25–N16 region. The top line represents the total map, the bottom line the B25–N16 region. The relative location of each site in the B25–N16 region is indicated by a dot or column of dots. The number of dots indicates the number of recurrences at that site. The filled areas in the map indicate genes as defined by the complementation tests. Each gene is identified by its reference *ts* marker. As an indication of scale, markers at the ends of the A13 gene give about 4 percent recombination.

at 45 sites within the region. (This number of sites is a minimum estimate since no attempt to detect recombination frequencies lower than 0.1 percent was made. Thus apparent repeats of mutations at the same site may represent, in some cases, mutations at different but closely linked sites.) As is shown in Table 1, the distribution of mutations at the various sites is close to random. There is no striking evidence of "hot spots," that is, sites at which mutations recur with high frequency. The distribution of the sites along the map is not uniform. The sites ap-

TABLE 1

Distribution of ts *mutations in the B25–N16 region*

Number of mutations per site	Observed number of sites	Calculated* number of sites
0	. .	41
1	30	(30)
2	11	(11)
3	1	2.7
4	0	0.5
5	2	0.07

Number of sites per gene	Observed number of genes	Calculated* number of genes
0	.	1.6
1	4	(4)
2	5	(5)
3	3	6
10	1	<0.01
11	1	<0.01

* Calculated from the Poisson distribution, using the observed ratio of Class 1 to Class 2 (values in parentheses) to compute the mean.

pear to be "clustered," with intervals of 5 percent to 10 percent recombination occurring between clusters.

All complementation tests between mutations in different clusters gave burst sizes which were about 50 percent that of the wild-type control. Burst sizes significantly less than this were found only for mutant pairs in the same cluster. Most of the clusters contain mutations at only a few different sites. In all of these cases, the burst sizes obtained from tests between mutations within the clusters were less than 10 percent of the wild-type controls although in many cases they were found to be significantly higher than that obtained with either mutant alone. The burst sizes obtained were reproducible within a factor of about two.

In two clusters, there are a large number of sites, the A10 cluster with ten sites and the A13 cluster with 11 sites. For illustrative purposes, the complementation and mapping results involving the various combinations of the mutations in the A13 cluster and the two adjacent clusters are presented in Table 2. The results with the A10 cluster are similar to those with A13. It is quite clear that while the majority of intracluster combinations gave burst sizes significantly less than 50 percent of the wild-type controls, in most cases the burst sizes were significantly higher than either of the two mutant types alone. For some pairs in the A10 cluster the burst sizes reach the 50 percent level found for combinations of mutants in different clusters, but the mutations which give this high-level of complementation give low burst sizes when in combination with other mutations in the same cluster.

In general, the mutations can be assigned to genes on the basis of these quantitative complementation tests. In most cases, mutations in the same gene give burst sizes considerably lower than 50 percent of wild-type controls when in combination with other mutations belonging to the same gene. However, most such combinations do exhibit partial complementation since the burst sizes obtained are usually much higher than those found for either of the two mutant types alone. The range of burst sizes is great. No attempt to construct complementation maps (see CATCHESIDE and OVERTON 1958) has been made since virtually every test is positive if compared to the single mutants alone.

It should be pointed out that some errors in assignment of mutants to genes may occur in cases where few sites are involved, since some combinations within functional groups do give burst sizes about 50 percent of wild type. Such cases might be interpreted as inter- rather than intragenic complementation.

The N1–N10 region: Mutants from the N1–N10 region which were obtained from the random isolation series were tested for recombination and complementation in many combinations. These tests included only those mutants whose functional relationships were not clear from the spot test results. With one exception, the complementation results were similar to those obtained from mutations in the B25–N16 region. Tests involving mutations located between N1 and A44 gave burst sizes about 50 percent of the wild-type control in most combinations rather than in very few combinations as was generally found for mutants located in the same functional group (see Table 3). However, all of the mutants in this region, but none elsewhere, gave low burst sizes when in combination with mutant A44.

TABLE 2

*Complementation and recombination in genes N6, A13 and N38**

	Gene N6		Gene A13												Gene N38	
	N6	*am*N93	B113	*am*N69	B105	B81	B92	B60	B1	A15	N40	B27	A13	A1	*am*N104	N38	N49
N6	**0.4**	*0.66*	2.3	..	2.1	2.7	..	..	2.8	..	..	..	5.1	..	5.3	11	..
*am*N93	*<0.3*	*<0.01*	*2.2*	..	..	*2.9*	..	*3.1*	*2.9*	..	..	..	*4.6*	..	*4.3*	*10*	..
B113	76	35	**<0.01**	..	0.25	0.69	0.97	1.4	1.5	1.3	3.1	3.4	2.8	3.4	..	6.7	..
*am*N69	*63*	..	*<0.3*	***<0.01***	*0.24*	*0.34*	*0.59*	*0.64*	*1.3*	*2.3*	*3.3*	*3.8*	*3.0*	*4.4*	..	*7.3*	..
B105	59	..	4.1	*<0.2*	**<0.01**	0.69	1.2	1.5	1.4	1.3	2.5	2.9	2.3	3.4	..	..	..
B81	*77*	*49*	18	*<0.2*	0.75	**0.12**	0.29	1.8	1.2	1.2	2.7	2.7	2.4	2.5	*2.5*	8.8	..
B92	..	..	21	*<0.2*	16	7.9	**0.35**	0.83	1.3	1.4	2.2	2.5	2.1	3.2	..	..	..
B60	..	*42*	4.6	*<0.3*	12	10	15	**<0.01**	0.77	0.82	2.1	2.1	1.5	2.3	*2.2*	6.3	..
B1	52	*34*	6.0	*<0.3*	13	15	20	6.4	**<0.01**	0.10	0.55	0.97	0.78	1.2	..	..	..
A15	..	..	1.6	*<0.3*	4.9	5.7	9.3	0.94	0.07	**<0.1**	0.51	0.55	0.6	1.1	..	..	..
N40	..	..	0.6	*<0.3*	1.4	2.7	2.8	0.7	0.42	0.5	**0.1**	0.25	0.28	1.1	*1.3*	4.2	6.6
B27	..	..	0.6	*<0.3*	0.67	0.86	1.1	0.21	0.22	0.04	0.01	**<0.01**	0.11	0.82	..	..	..
A13	50	*33*	0.07	*<0.3*	0.11	0.49	0.95	0.03	0.10	0.1	0.1	0.02	**<0.1**	0.6	*1.2*	5.4	7.1
A1	..	..	3.4	*<0.4*	15	8.3	11	2.9	4.4	1.0	0.1	0.06	<0.1	**0.3**	..	4.7	5.3
*am*N104	*64*	*27*	*0.02*	..	..	*0.08*	..	*0.02*	*<0.01*	..	*<0.01*	..	*<0.01*	*0.02*	***<0.01***	*6.1*	..
N38	72	*43*	45	*36*	..	53	..	45	..	65	42	..	100	55	*44*	**0.1**	1.8
N49	..	..	..	..	..	..	..	..	..	60	62	..	80	75	..	2.0	**<0.1**

* Burst size measurements are presented below the diagonal. They are expressed as percent of the wild-type control. Burst sizes (also percent of wild type) of the mutants alone are given on the diagonal (in boldface type). Above the diagonal are given recombination percentages for the appropriate marker pairs. Measurements involving amber mutants are italicized.

On the basis of this result, we conclude that this region is one gene although complementation between many members of this group is very efficient. Further evidence supporting this conclusion comes from studies involving *am* × *ts* combinations described below.

The A41–L86 region: Detailed complementation and recombination studies were also performed with mutants located in the region bounded by the mutations A41–L86. Phage carrying *ts* mutations located in this portion of the genome are characterized by defects in DNA synthesis and also in the lytic mechanism (EPSTEIN *et al.* 1963) during growth at the high temperature. Many of these "early" mutants are arrested in growth by incubation at the high temperature since they can resume growth and phage production if transferred to lower temperatures after the "normal" latent period would be completed. In contrast, the mutants defective in genes in other regions of the genome induce lysis of the infected bacteria at the same time as wild type. To make the complementation tests with the mutants of the A41–L86 region comparable to those involving the other mutants, it was found necessary to insure the lysis or death of the complexes at normal lysis time by the addition of chloroform to the growth tubes. In other respects, the complementation tests were identical to those described earlier. No ambiguity was found in the assignment of mutations to genes, although here, as in the other regions studied, some degree of intragenic complementation appeared to be the rule rather than the exception.

Complementation tests. ts × am: EPSTEIN *et al.* (1964) found that with *am* mutations complementation does not occur between any members of the same gene. Thus, in contrast to the *ts* mutations, no intragenic complementation is observed with *am* mutants. It is therefore of interest to investigate the complementation behavior of combinations involving *am* and *ts* mutants. Such tests also give information as to which genes contain both *am* and *ts* mutations.

Preliminary tests consisted of complementation spot tests between representative *am* and *ts* mutants from each of the genes found for each system. No modification of the spot test procedure was necessary since the tests are performed on strain S/6 at high temperature. *ts* mutants cannot grow at high temperature and *am* mutants cannot grow on S/6 (a derivative of B), thus any clearing in the overlayed spots indicates that complementation occurs between the *ts* and *am* mutants tested. In this way a number of different *am* × *ts* pairs were found not to complement, indicating that both types of mutations can reside in the same gene. As a further check, most negative tests, and all tests involving mutations in adjacent regions of the map, were repeated by the burst size method. No modification of the procedure was necessary except that measurements of total yield were made on plates seeded with strain CR63, and incubated at low temperature, conditions permissive for both mutant types.

On the basis of these complementation tests, it was found that 26 genes contain both *am* and *ts* mutations. Mutations from the remaining *am* and *ts* genes showed complementation with one another in all combinations tested. The results of these tests are summarized in Figure 2. Since some of the genes we have identified contain both *am* and *ts* mutations, while others contain only *ts* or only *am* mutations,

TABLE 3

*Complementation and recombination in genes A44 and N30**

	Gene A44												Gene N30	
	N1	B3	B111	A20	A51	B41	B22	*am*N58	B33	B104	B45	B63	A44	N30	*am*B252
N1	0.03	4.4	..	8.1	7.2	8.2	..	*12*	..	..	17	..	17	21	..
B3	10	0.06	2.1	5.7	6.7	..	..	*13*	12	19	20	..	25	..	..
B111	65	28	0.005	2.4	5.2	4.3	7.6	*9.6*	7.5	12	15	..	25	..	..
A20	15	6	20	0.017	3.1	..	9.7	*9.9*	13	16	18	26	21	24	..
A51	100	50	100	58	0.32	1.6	..	*8.3*	10	12	15	20	23	..	..
B41	42	34	64	13	0.09	0.016	..	*7.4*	..	15	..	20	23	..	..
B22	45	36	7	27	88	75	0.094	*3.0*	..	..	..	14	16	..	..
*am*N58	*0.8*	*0.9*	*0.6*	*0.11*	*1.2*	*1.2*	*0.12*	*<0.01*	*4.9*	*7.6*	*14*	*14*	*13*	*16*	..
B33	30	17	28	11	77	52	55	*0.12*	0.02	..	..	..	..	..	..
B104	38	23	29	28	43	67	68	*0.09*	45	0.001	15	15	16	..	..
B45	41	25	50	28	100	70	62	*0.33*	49	53	0.002	2	..	24	..
B63	..	53	..	17	74	80	77	*0.36*	29	30	0.45	0.3	10	20	..
A44	0.7	3.5	2	0.3	3.7	3.0	1.5	*0.05*	0.28	0.32	0.28	1.6	<0.01	19	*25*
N30	58	59	40	39	79	72	61	*23*	43	47	52	77	48	<0.01	*11*
*am*B252	..	..	..	..	..	..	..	..	..	..	..	..	*37*	*0.05*	*<0.01*

* See footnote to Table 2.

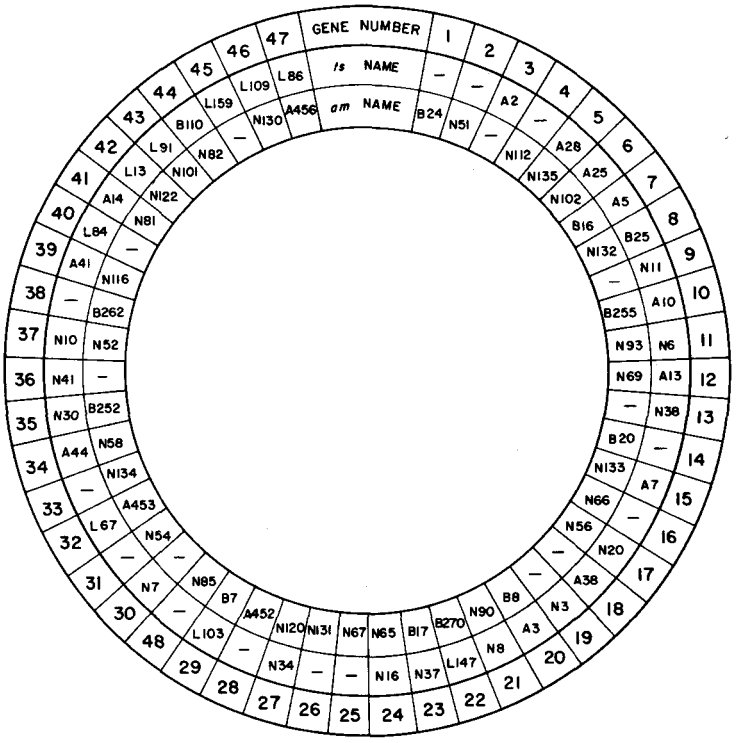

FIGURE 2.—The correspondence between *am* and *ts* genes. The genes are arranged in their relative order around the map. Each gene is assigned a number. The sectors demark independent genes; thus *am* and *ts* mutants in the same sector are noncomplementary. The specific *am* and *ts* mutants indicated are the reference markers for each gene as given in EDGAR and LIELAUSIS (1964) and EPSTEIN *et al.* (1964). The numbers of the genes were assigned prior to the publication of EPSTEIN *et al.* 1963. It has since been found that the gene referred to as gene 29 in that publication is in fact two genes, now designated 29 and 48.

we have assigned to each identified gene a number independent of its previous *am* or *ts* designation. This numbering system is also given in Figure 2.

Since mutations in the same gene should be closely linked, we may use noncomplementation as a criterion for close linkage and thus compare the published maps of the *ts* mutations (EDGAR and LIELAUSIS 1964) and of the *am* mutations (EPSTEIN *et al.* 1964). Such a comparison shows remarkably good agreement for both order of and recombination distances between corresponding genes. The few discrepancies may well be due to the fact that, since many genes have appreciable genetic length, the mutations used in the construction of the two maps may be at different locations within the gene. A composite map is given in Figure 3. The recombination distances given in Figure 3 are in most cases approximate averages of the distances obtained from the *ts* map and the *am* map. Some distances are from the *ts* map only, others from the *am* map. In many cases recombination distances between *am* and *ts* mutants have been measured and are in accord with the map presented.

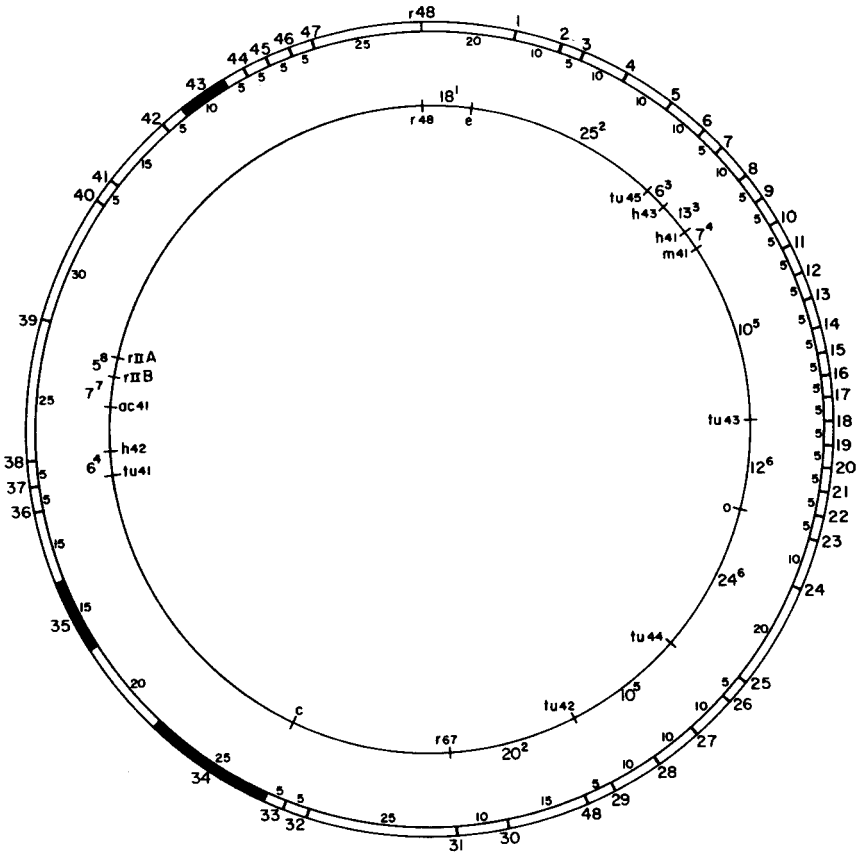

FIGURE 3.—A composite map of T4D. The outer circle is a map of the genes containing conditional lethal sites. Genes with appreciable genetic length are indicated by the filled areas in the map. Approximate recombination values are indicated within the circle. The map has been drawn only roughly to scale. The inner circle is a map of standard markers. Their assigned location, determined largely by the crosses given in Table 3, indicates their approximate position relative to the conditional lethals. The recombination values between adjacent standard markers are given in the figure. The superscripts indicate the sources of these recombination values as follows: *Superscript* 1: EDGAR and LIELAUSIS (1964); 2: EDGAR and LIELAUSIS (unpublished); 3: JINKS (1961); 4: EDGAR (1958); 5: DOERMANN and HILL (1953); 6: BRENNER and BARNETT (1959); 7: EDGAR and EPSTEIN (1961); 8: DOERMANN (1953).

The results of a few of the burst size measurements of complementation between *am* and *ts* mutations are included in Tables 2 and 3. A most interesting result is that no evidence was found for intragenic complementation in *am* × *ts* combinations. That is, the burst sizes found were either about 50 percent of wild type, for combinations of mutations in different genes, or were very close to the values obtained for the parental types alone in tests involving *am* and *ts* mutants in the same gene. The subdivision of the *ts* mutants into genes on the basis of the *ts* × *ts* tests was, without exception, in accord with the results of the *ts* × *am* tests. For example (Table 3) *am*N58 does not complement with any of the *ts* mutants

in segment N1–A44 which had been called one gene on the basis of the *ts* × *ts* tests. This *am* mutant does, however, complement fully with *ts* mutants outside of this region.

The correspondence of the conditional lethal map to the standard map: A number of mutations of T4D other than *am* and *ts* are known and have been mapped. It is thus of interest to know the location of these mutations relative to the genes which we have uncovered with the conditional lethals. A number of crosses involving these mutations and the *am* or *ts* mutations have been made. These, in conjunction with published mapping data, permit the construction of a "comparative map" (shown in Figure 3). Data from crosses between these "standard" markers and the conditional lethal markers used in constructing the map in Figure 3 are given in Table 4. Recombination distances involving crosses between conditional lethals and various *r* markers have been presented elsewhere (EDGAR and LIELAUSIS 1964; EPSTEIN *et al.* 1963).

The location of the *c* or cofactor requirement locus was determined by MR. R. D'ARI, who isolated and mapped several cofactor requiring mutants of T4D. One of these was also *ts* in phenotype and fell in gene 34 by complementation tests. In many instances, the location of the standard markers relative to the conditional lethals is known only roughly since the available crosses involve rather distantly linked markers only.

TABLE 4

*Crosses of standard markers by conditional lethals**

Parents			
	Conditional lethal		
Standard marker	Mutant	Gene No.	Percent recombination
*tu*45a	*ts*A25	6	0.5[2]
	*am*B16	7	5.8[3]
*h*43	*am*B16	7	2.7[2]
*h*49	*am*B16	7	11
*h*41	*am*B16	7	16[2]
*tu*43	*am*B17	23	11[2]
*tu*44	*am*B17	23	22[3]
	*am*B7	29	9.5[4]
	*am*N85	48	11[2]
*tu*42	*am*B7	29	6.8[3]
	*am*N85	48	3.3
	*am*N54	31	19
*r*67	*am*B7	29	21[2]
	*am*N85	48	15
	*am*N54	31	1.4
*C*10	*ts*N1	34	5.2
	*ts*A20	34	4.8
*tu*41	*am*N52	37	0.5
	*am*N62	38	12

* The number of crosses upon which the average recombination percentage is based is indicated by a superscript.

DISCUSSION

We have presented results concerning the distribution of *ts* mutations at various sites within the genome of T4D. The analysis of the B25–N16 region constitutes an exhaustive analysis of a random sample of mutants of independent origin. It was found that the 66 mutations studied were located at a minimum of 45 sites within the region. The mutations appeared to be randomly distributed among the sites with little indication of hot spots. From the distribution of mutations among sites, it would appear that the sites observed constitute about one half of the total obtainable *ts* sites in this region (see Table 1). This region is at least eight times longer (in recombination units) than the *rII* region. Since the *rII* region contains at least 300 mutable elements (BENZER 1961), it follows that the B25–N16 region probably contains many thousands of mutable elements. Thus the *ts* mutable sites must constitute only a very small fraction of the total number of mutable sites in this region.

The sites do not appear to be uniformly distributed within this region, but appear to be clustered. This clustered distribution of sites suggests that certain genes or certain portions of genes are not susceptible to mutation to temperature sensitivity. This fact is also apparent if one considers the distribution of sites per gene. Two of the 14 genes (10 and 12) in the region contain about one half of the total sites found.

We conclude that in this particular region of the genome only a fraction of the total mutable sites are subject to mutation to give a temperature-sensitive phenotype and that these sites are not distributed uniformly throughout the region. This finding supports the idea that the temperature-sensitive phenotype is a site specific rather than a gene specific property.

The complementation properties of mutations of various genes in other organisms such as Neurospora and *Escherichia coli* have been extensively studied (see, for example, CATCHESIDE and OVERTON 1958; WOODWARD 1960; GAREN and GAREN 1963; SCHLESINGER and LEVINTHAL 1963). In general, it is found that two classes of mutations are found. These classes of mutations have been designated as "complementing" and "noncomplementing." Complementing mutants complement with at least some other mutants of this class within the same gene. In general, the degree of complementation found is less than that observed for mutations in different genes. Noncomplementing mutants complement neither with each other nor with the complementing mutants of the same gene. Both types of mutations can be point mutations and may be located anywhere within the gene. The available evidence suggests that complementation within a gene is due to the formation of hybrid dimers or aggregates of higher order containing polypeptide chains of both mutant types. Aggregates of each mutant type alone are inactive while the hybrid aggregate is active (see WOODWARD 1960; SCHLESINGER and LEVINTHAL 1963). It has been shown that many noncomplementing mutants induce the synthesis of little, if any, protein related in structure to that produced by the nonmutant gene, while many complementing mutants produce normal levels of defective protein. While this correlation is not perfect, it would appear that the noncomplementing nature of many of these mutants reside in the fact that they are "nonsense" mutations, incapable of inducing the synthesis

of normal levels of gene product. Complementing mutants, however, may be thought to be "missense" mutations resulting in production of normal levels of a gene product which is, however, inactive.

Our results on the complementation behavior of *ts* and *am* mutants fits well with this pattern of intragenic complementation observed in other organisms. It would seem that most, if not all, *ts* mutations behave as complementing mutants. *am* mutants, on the other hand, behave as noncomplementing mutants. Physiological evidence also suggests that *ts* mutants are most likely missense mutants and *am* mutations are most likely nonsense mutations (EPSTEIN *et al.* 1964). A class of mutants similar to the amber mutants, suppressor-sensitive mutants of the alkaline phosphatase gene of *E. coli*, has also been shown to consist of noncomplementing mutants and to be incapable of forming normal levels of gene-product protein in the absence of the suppressor (GAREN and SIDDIQI 1962). Thus our results on the complementation behavior of the two classes of mutations support this "missense-nonsense" interpretation of the effects of the two types of mutations upon the activity of the genes of the phage. Further consideration of the nature of amber mutations is given by EPSTEIN *et al.* (1964).

Since our results are in accord with the results of complementation studies of genes of other organisms, we are led to believe that our subdivision of the genome of the phage into genes is a satisfactory one.

It should be noted that SÉCHAUD and STREISINGER (1962) have demonstrated that recombinants arising in the vegetative pool can express their phenotype. Thus it might be thought that our complementation results reflect not intragenic complementation as it occurs in other organisms but the occurrence of recombination between the two mutants tested and the subsequent phenotypic expression of the recombinants. However, the complementation patterns we observe are not obviously correlated with the amount of recombination found for the corresponding pairs of markers. Within the same cluster, tightly linked markers may exhibit more complementation than distantly linked ones. As an extreme example, in gene 34 the *ts* mutants A44 and B3 result in the production of 10 percent wild-type recombinants, whereas the degree of complementation is less than 3 percent of wild type. In the same gene the mutants B111 and A20 give only 2.4 percent recombination but 28 percent complementation.

These results do not conflict with those of SÉCHAUD and STREISINGER, however, since the effect of recombination on the production of wild-type phenotype which they reported is quantitatively much less than that which we observe in our complementation studies. Thus we believe that the effects of recombination on the complementation pattern are minor.

Our comparative analysis of the *am* and *ts* mutations shows that in many cases *am* and *ts* mutations occur within the same genes. This reinforces our contention that the *am* and *ts* mutations may occur at specific sites located in a wide variety of genes. The combined analysis shows that *ts* and *am* mutations occur in at least 48 genes of the phage. It is probable that the collection already obtained identifies a significant fraction of the total gene complement of the phage. However, the analysis is by no means exhaustive and it is likely that mutations of both types in many more genes can be obtained.

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SUMMARY

The results of complementation tests involving temperature-sensitive (*ts*) and amber (*am*) mutations of bacteriophage T4D are described. Intragenic complementation occurs between many *ts* mutations in the same gene, but not between *am* and *ts* mutations located in the same gene. These results suggest, on the basis of comparable studies with other organisms, that *am* mutations are of the non-complementing type and are "nonsense" mutations, while *ts* mutations are of the "complementing" type and thus are "missense" mutations. A composite map of T4D combining *ts*, *am* and other known mutations is presented.

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