THE CIRCULAR LINKAGE MAP OF BACTERIOPHAGE T2H[1]

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THE purpose of this paper is to report extensive linkage data obtained with host range (*ht*) mutants which confirm the circular nature of the T2 coliphage genetic map. The genetic map of T2 was once considered to be two independent linkage groups until STREISINGER and BRUCE (1960) demonstrated its unity. Although the experimental evidence was not published until 1964 (STREISINGER, EDGAR and DENHARDT 1964), STREISINGER, in 1961, first suggested a circular genetic map as the explanation of the results of certain crosses with T4 mutants. Extensive studies (EPSTEIN *et al.*, 1963; EDGAR, DENHARDT, and EPSTEIN 1964; and EDGAR and LIELAUSIS 1964) using many temperature sensitive (*ts*) and amber mutants (*am*) showed that in T4 no two *ts* and *am* sites are sufficiently widely separated to be termed unlinked. Foss and STAHL (1963), using rescue experiments, showed that the order of four distantly linked markers in T4 could be arranged on a circular map. The best interpretation of all these data is a circular linkage map.

BAYLOR and SILVER 1961, showed that certain *ht* mutants were mutations of genes which determine some of the proteins of the mature phage particle. Their experiments were designed to distinguish whether the gene directly determined a structure or modified the product of another gene. To extend this work on mechanism of gene control, we applied the same tests to other parts of the genome which contained heat sensitive *ht* mutants. We feel the results of these experiments make it likely that *ht* mutants occur only in genes which directly control the structural proteins of the phage.

The *ht* mutants control the adsorption of the phage to the resistant cell B/2 and affect the heat stability of the mature phage as well. Each of these properties exhibits phenotypic mixing and may be characteristic of any part of the phage external protein. The different mutants must be located in genes which determine different structures of the phage because they are distributed over two thirds of the linkage map.

MATERIALS AND METHODS

Bacterial strains: The following strains of *Escherichia coli* were employed. H (Hershey), Bg (STREISINGER), and B/1,5 (LURIA) were used as host bacteria for crosses. S (HERSHEY) was used for plating. B/2H (HERSHEY's 2bc) served as an indicator strain for plating and as recipient cells for plaques transferred by velvet from S-grown master plates. K12λ (TESSMAN) was used in crosses involving *rII* mutants.

Phages: These were all derived as single-step mutants from T2H (HERSHEY).

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Mutant markers: r1, r2, r7, r13 (rapid lysis), and *h* (host range) were obtained from HERSHEY. rm1, a new *r* linking r1 and r7, was isolated during this study. The quinacrine resistant mutant, *q*, and the proflavine resistant mutant, *pr*, were isolated from T2H by HESSLER (1963). The *ts*1, *ts*5, and *ts*6 mutants (temperature sensitive during development of the infected complex) were isolated during this study.

Media: Standard commercial media were used for bacterial and phage growth. Bacto-tryptone, tryptone broth, Bacto-agar, T2 phosphate buffer (pH 7.0), M9 synthetic medium and acetate buffer (pH 5.2) were employed. See ADAMS (1959) for standard formulae.

Replication: Plaques plated on one cell were transferred by velvet to a different cell (BAYLOR, HURST, ALLEN and BERTANI 1957) to determine the host range phenotype.

Isolation of mutants: Each mutant was isolated from the progeny of a single wild-type plaque to insure that each mutant resulted from a different mutational event.

The *ht* mutants 2 through 14 and 31 through 59 were obtained by replicating many plaques to B/2 and selecting any master plaques which gave a turbid copy plaque (wild type does not transfer; the *h* mutant gives a clear copy plaque). Mutants 15 through 30 were selected by plating on B/2 after preadsorption onto ultraviolet-treated B cells. Preadsorption increases the efficiency of plating on the indicator cell and eliminates phenotypic mixing.

The *ts* (temperature sensitive) mutants were detected after proflavine or HNO_2 treatment by incubating assay plates at 30°C and transferring by velvet to new plates which were then incubated at 42°C. Those plaques which did not grow at 42°C were isolated from the master plate.

HNO_2 mutagenesis: Samples of either free phage or infected complexes were treated for 2 minutes in 0.05 M KNO_2 in acetate-acetic acid buffer 0.2 M (pH 4.0) at room temperature (BAYLOR and MAHLER 1962). This 2-minute HNO_2 treatment corresponds to about one hit per free phage.

Acridine mutagenesis: Samples of infected complexes were treated with 4.0 μg/ml proflavine, according to ORGEL and BRENNER (1961). Mutants were detected as turbid transfers on B/2 or by direct plating on B/2 after preadsorption on B.

Crosses: Crosses were made as described by BAYLOR *et al.* (1957). Cells were infected with three to five of each parental phage type. The multiplicity of infection was determined by the ratio of surviving cells to the initial uninfected cells. Adsorption proceeded in buffer salts without nutrient. After 30 minutes of development in broth, the infected complexes were chloroformed to release any residual intracellular phage, and the progeny were assayed. Phenotypes of the progeny of genetic crosses between two *ht* mutant parents were distinguished after velvet transfer of the mature plaques on the sensitive cell to the resistant cell (B/2). When this was done the parental *ht* types formed very turbid plaques on B/2 and the double *ht ht* recombinants usually formed less turbid plaques than either parental type. By contrast the wild-type recombinant plaques did not transfer to B/2.

Calculation of correction factors for crosses: The majority of the crosses of *ht* mutants were of the general plan: r1 *ht*a × r13 *ht*b. The production of r^+ recombinants between parental r1 and r13 provided an internal control permitting the calculation of a correction factor for the recombination frequency of the two *ht*'s. It had been estimated previously (BAYLOR *et al.*, 1957) that the maximum recombination between r1 and r13 is 45 percent. The recombination between r1 and r13 in each cross was taken as twice the number of r^+ recombinants divided by the total cross progeny, because r1 r13 recombinants are undetectable. The r1-r13 linkage was then corrected to 45 percent, and a correction factor was thus established for each cross. The *ht*a-*ht*b recombination frequency was taken as twice the number of *ht*a$^+$ *ht*b$^+$ divided by total cross progeny. This latter figure was then corrected by the amount required to alter the internal r1-r13 linkage to 45 percent. The detection of double *ht* recombinants was not always feasible in crosses between two *ht* mutants but when *ht*a *ht*b recombinants were found, they were considered in calculating the *ht*a-*ht*b linkage.

Use of three-factor crosses: The region between *ht*4 and *ht*13 was first mapped using *ht* × *ht* crosses, but this simple cross did not reliably permit us to infer the order of the closely linked *ht* markers. To improve the reliability of the order, several *ht* mutants were individually crossed

to the double mutant *ts*1 *q*1. The region between *ts*1 and *q*1 includes *ht*4 and *ht*13. The progeny of the three-factor cross were plated on agar supplemented with quinacrine (10 μg/ml). Incubation at 42°C screened out all *ts*-carrying progeny. On the quinacrine plates, any progeny carrying the *q* marker gave a normal plaque, but the q^+ forms gave distinctly smaller star-shaped plaques. Then to distinguish the *ht* phenotype, the plaques on quinacrine were transferred by velvet to B/2 cells on plain agar. The ratio of the recombinant *q* ht^+ ts^+ to all detectable recombinants recovered in each cross gives the distance from *ts*1 to the particular *ht* marker being tested. The *ht* to *ts*1 distance measured in this fashion is not directly comparable to other genetic distances, but the two outside markers permit us to infer more accurately the order of the mutants (Table 3).

Origin of the mutants: About one half of the *ht* mutants mapped were spontaneous in origin and occurred with a frequency of 2×10^{-4}. The mutation frequency was the same whether determined by the nonselective method (velvet transfer of plaques from the sensitive cell to the resistant cell) or by the selective method (direct plating on the resistant cell after preadsorption on the sensitive cell). Preadsorption increases the detectable number of *h* mutants by a factor of ten and the number of *ht* mutants by a factor of 100 because phenotypic mixing is eliminated and efficiency of plating is enhanced. Most mutants obtained by the nonselective method were not mixed with wild type.

The number of *ht* mutants detected after a nitrous acid treatment which corresponded to one hit per phage was about five times that in the untreated sample (see BAYLOR and MAHLER 1962). About two thirds of these *ht* mutants were heterozygous, that is, the mutant plaque contained both mutant and wild-type phage.

An increased number of both *ht* and *ts* mutants were found after treatment of wild-type stocks with proflavine, and the question of whether or not they were induced by proflavine is under investigation.

RESULTS

The circular map: The isolation of new mutants has enabled us to demonstrate linkage between *r*1 and *r*7 and between *ht*6 and *ht*8. These four markers had been the terminal markers of two previously separate linkage groups. To make the map, new mutants were routinely crossed with certain reference *ht* markers (those inside the circle, Figure 1) to find their approximate location and then were crossed with one another to establish closest neighbors. The results from many different crosses not only between neighbors ($a \times b$ and $b \times c$) but also between outside markers ($a \times c$) permitted us to arrange the data in a sequence. Once the markers were put into a sequence, a map was drawn spacing the markers according to the recombination distances found between adjacent markers. The data are best described by a circular map (Figure 1).

The data of Table 1 from which the map was prepared have been condensed from several hundred crosses. To show the distances between markers we have selected for Table 1 only the linkage data between adjacent or nearly adjacent markers.

The order of the sequence which closes the circle from *h* to *r*1 (*h*-*r*7-*r*m1-*r*1) was established by the crosses shown in Table 2. The marker *r*m1 was more closely linked to *r*1 than was either *r*7 or *h*, and it was more closely linked to *r*7 than to *h*. These very long linkages were revealed only in premature lysates because differences between large recombination distances become less significant as genetic equilibrium is approached (STREISINGER and BRUCE 1960).

Distribution of ht *markers:* The *ht* markers are distributed clockwise from *r*1 to the *rII* region, occupying approximately two thirds of the perimeter. There is

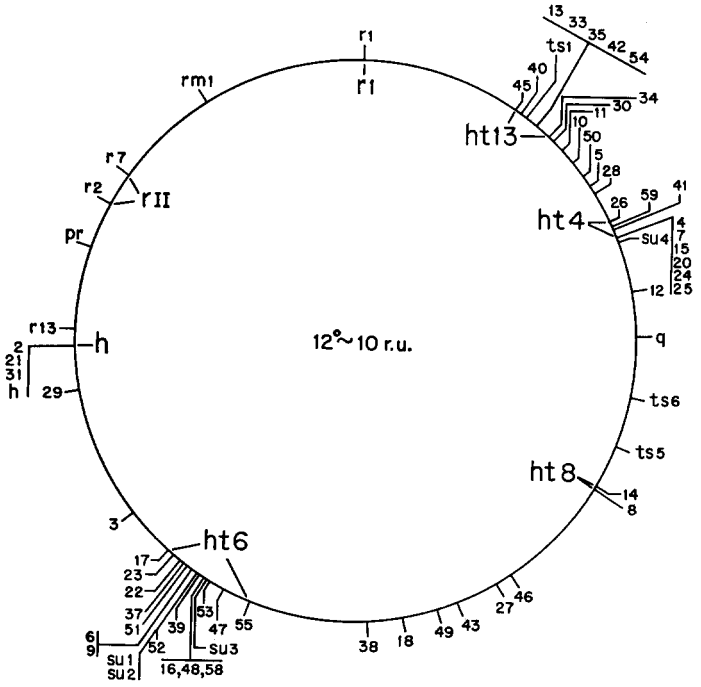

FIGURE 1.—Linkage map of T2H constructed from data in Tables 1, 2, and 3. The numbers are designation of the various *ht*'s. r = rapid lysis, q = quinacrine resistant, *pr* = proflavine resistant, *ts* = temperature sensitive, *su* = suppressor of *ht*. Regions referred to in the text are marked inside the circle. The *r*1, *rII*, *pr* and *q* markers are probably homologous to *r*48, *rII*, *ac*, and *q* in T4.

little evidence of strong clustering except at *h* and at *ht*4. Forty-four percent of the mutants map between *ht*13 and *ht*4 and 30 percent map near *ht*6. About 20 percent are scattered and well separated from one another.

The tentative order of the mutants between *ht*13 and *ht*4 was established by *ht* × *ht* crosses. This order was confirmed by *ts*1 *q*1 × *ht* crosses with nine out of 12 *ht* mutants within the region (see Table 3). The other three *ht* mutants gave aberrant results in these crosses and require further study.

The distribution of the mutants in the region between *ht*4 and *ht*13 may depend on whether or not a mutagenic agent was employed. Six of the 12 spontaneous mutants are clustered within 1 percent of *ht*4. Eight of the ten HNO_2-induced mutants are within 5 percent of *ht*13.

The T2H genetic map and the T4D genetic map may be compared in Figure 1. The *ht* mutations, all of which are concerned with external phage proteins, are distributed in analogous positions to the *ts* and *am* mutants of T4 which have been shown to affect "late functions."

Suppressors of host range mutants: Apparent revertants to the wild-type (ht^+) phenotype were observed occasionally in *ht* stocks. This reversion to a decreased host range was more easily observed when the double *ht*(*ht ht*) mutant was

TABLE 1

Map distances between markers of T2H. Data are presented from only those crosses which confirm the linkage of adjacent markers

Parental genotypes	Number of plaques	Percent recombination (corrected)*	Parental genotypes	Number of plaques	Percent recombination (corrected)
*r*1 × *rm*1	400	18.4			
*rm*1 × *r*7	665	18.2	*ht*6 × *ht*52	207	1.3
*r*7 × *r*2	396	8.0	× *ht*39	228	3.1
*r*7 × *pr*	967	13.7	× *ht*16	233	4.3
pr × *r*13	718	14.2	× *ht*48	50	<4.4
pr × *h*	718	14.6	× *ht*58	138	4.6
*r*13 × *h*	718	3.6	× *ht*53	556	6.4
*ht*2 × *r*13	1446	1.5 ± 0.3†	× *ht*47	725	10.2 ± 6.0
*ht*21 × *ht*2	863	<0.4	*ht*53 × *ht*47	349	3.2
*ht*29 × *ht*2	556	7.2	*ht*55 × *ht*47	318	8.5
*ht*31 × *ht*2	912	0.3	*ht*55 × *ht*6	1313	10.4 ± 4.5‡
*ht*3 × *ht*2	1057	22.0 ± 1.9	*ht*47 × *ht*38	553	21.0
*ht*3 × *ht*6	1547	14.1 ± 0.4	*ht*38 × *ht*55	350	18.5
*ht*6 × *ht*17	379	4.7	*ht*38 × *ht*18	158	5.7
× *ht*23	794	4.1	*ht*38 × *ht*49	249	11.4
× *ht*22	641	3.4	*ht*49 × *ht*43	445	4.0
× *ht*37	810	2.4	*ht*43 × *ht*27	166	7.2
× *ht*51	320	<0.9	*ht*27 × *ht*46	294	4.8
× *ht*9	664	<0.2	*ht*46 × *ht*43	218	21.9
*ht*46 × *ht*8	457	21.7 ± 3.4	*ht*50 × *ht*4	270	7.9
*ht*8 × *ht*14	889	<0.25 ± 0.16	*ht*50 × *ht*13	213	9.0 ± 0.1
*ht*8 × *q*	250	21.0	*ht*5 × *ht*10	577	3.5
*ts*5 × *q*	309	18.0	*ht*10 × *ht*11	1193	2.0
*ts*5 × *ts*6	500	17.0	*ht*34× *ht*5	680	10.1
*ts*6 × *q*	291	9.3	*ht*34 × *ht*13	275	2.6
q × *ht*12	212	7.1	*ht*28 × *ht*13	411	3.9
*ht*12 × *ht*7	1309	9.2 ± 2.2	*ht*33 × *ht*34	1192	2.5 ± 0.05‡
*ht*7 × *ht*4	2658	0.9 ± 0.06‡	*ht*54 × *ht*13	296	<0.8
*ht*4 × *ht*20	892	0.7	*ht*35 × *ht*33	875	1.1
*ht*4 × *ht*24	723	0.9	*ht*40 × *ht*13	695	5.2 ± 2.8‡
*ht*25 × *ht*20	557	<0.4	*ht*45 × *ht*13	210	3.2
*ht*24 × *ht*26	292	4.0	*ht*13 × *ts*1	387	3.9
*ht*15 × *ht*4	296	0.8	*ts*1 × *r*1	387	22.5
*ht*41 × *ht*4	943	2.7 ± 1.0			
*ht*59 × *ht*7	565	2.5			
*ht*7 × *ht*13	388	24.8			
*ht*5 × *ht*7	290	8.2			

* See MATERIALS and METHODS for explanation of calculation of correction factor for each cross.
† Except where indicated ‡ all standard errors are for two cases. Four cases are indicated by ‡.

used. Stocks of double *ht* mutants plated and transferred by velvet to B/2 contained a few plaques which gave a more turbid transfer than *ht ht*. The spontaneous frequency of these reduced host range mutants was 5×10^{-4}, approximately that of the forward mutation rate. Either of two mutational events could have occurred: (1) one of the two *ht* loci in the double mutant could have

TABLE 2

Location of rm*1 between* r*1 and* r*7 of T2H by three-factor crosses**

Cross A: rm1 $h \times r1$	Order inferred: r1–rm1–*h*	
Recombinant sites	Number of recombinants	Percent recombination
*rm*1–*r*1	74	18.4
*rm*1–*h*	46	11.4
*r*1–*h*	120	30.0
Total progeny: 400	$r+h : r+h+$ ratio: 1:5	
Cross B: rm1 $h \times r7$	Order inferred: rm1–*r*7–*h*	
Recombinant sites	Number of recombinants	Percent recombination
rm1–*r*7	122	18.2
rm1–*h*	176	26.5
*r*7–*h*	54	8.2
Total progeny: 665	$r+h : r+h+$ ratio: 6:1	

*Genotype	Phenotype of plaque transferred to K12λ
*r*1	clear
*rm*1	turbid
*r*7	blank

Although both crosses were performed simultaneously and growth was interrupted prematurely (12 minutes) by adding chloroform, Cross B had proceeded further towards genetic equilibrium. Nevertheless, the data substantiate the order derived from several other crosses which ran a normal growth cycle: *r*1–*rm*1–*r*7–*h*.

reverted to wild type or (2) a suppressor mutation could have occurred and acted on one or both of the *ht* loci. To test the hypothesis four turbid plaques were picked and made into stocks. Crosses between these stocks and wild type produced a few progeny which gave clear transfers to B/2 similar to the original double mutant. Therefore the reduced host range mutants seemed to contain the original *ht* markers plus a suppressor site.

Crosses were then performed to locate the suppressor site genetically in refer-

TABLE 3

Estimation of map order of ht *markers between* q *and* ts*1 by three-factor crosses:** r*1* ts*1* q*1* ht$^+$ × r*1* ts$^+$ q$^+$ ht

Mutant tested	Parental q^+ *ht*	Recombinant types			Ratio *q ht*$^+$/ total recombinants
		q ht	*q ht*$^+$	q^+ *ht*$^+$	
*ht*13	734	278	25	18	7.8
*ht*34	631	317	29	7	8.2
*ht*42	629	322	30	10	8.3
*ht*30	404	58	6	3	9.0
*ht*10	438	72	13	6	14.3
*ht*50	497	98	27	21	18.5
*ht*28	609	37	13	6	23.6
*ht*7	1064	315	253	30	41.7
*ht*12	731	25	70	12	65.4

Plates grown at 42°C on 10 μg/ml quinacrine agar; plaques transferred then by velvet to B/2.

* These data show the relative frequency of recombination between the *ts*1 and *ht* sites among the *ts*$^+$ progeny only and represent the relative order of the mutants between *ts*1 and *q*1 such that *ts*1 × *ts*1 = 0.0 percent and *ts*1 × *q*1 = 100 percent.

TABLE 4

Origin, phenotype, and location of suppressors of* ht

Source genotype	Mutation frequency	Suppressor mutants
*r*1 *ht*6 *ht*13	5×10^{-4}	*r*1 *ht*6 *ht*13 *su*1
		*r*1 *ht*6 *ht*13 *su*2
		*r*1 *ht*6 *ht*13 *su*3
*r*7 *ht*3 *ht*4	5×10^{-4}	*r*1 *ht*3 *ht*4 *su*4

Crosses

Parental genotypes	Recombinants (percent) 2(*r*+)	2(*ht*+)
*r*1 *ht*6 *ht*13 *su*1 × *r*13 *ht*6	33.8	33.0
× *r*1 *ht*13	. . .	2.2
*r*1 *ht*6 *ht*13 *su*2 × *r*13 *ht*6	>25	44.5
× *r*1 *ht*13	. . .	5.7
*r*1 *ht*6 *ht*13 *su*3 × *r*13 *ht*6	37.0	38.0
× *r*1 *ht*13	. . .	0.54
*r*7 *ht*3 *ht*4 *su*4 × *r*7 *ht*4	. . .	50.0
× *r*1 *ht*3	41.0	6.4

*

Genotype	Phenotype of plaque transferred to B/2
ht ht	nearly clear
ht ht+	turbid
ht ht su	very turbid
ht+ *ht*+	blank

ence to the two *ht* loci of the parent stock. The origin of the four mutants, the crosses performed, and the phenotypes of the recombinants are presented in Table 4. The genotypes of the recombinants were confirmed by backcrosses. In each case, the suppressor was closely linked to one of the two mutant *ht* markers. In the three crosses which contained *ht*6 and *ht*13, the recombination figures suggested that the suppressor was within the *ht*6 cluster. The suppressor isolated from *ht*3 *ht*4 appeared to be within the *ht*4 site.

Crosses of the suppressed mutants (*ht su*) with *h* gave unique recombinant particles (*h ht su*) which could be distinguished from *h* on transfer to B/2 by a slightly increased turbidity of the plaque. The suppressors therefore acted to reduce the efficiency of transfer of all host range phenotypic combinations tested.

Properties of the ht *mutant. Reaction of mature phage to heat:* Mutational changes in host range (*h* and *ht*) are often associated with simultaneous changes in the resistance of the mature phage to elevated temperatures. The mutants are of two classes: those like T2H which retain 10 percent of their initial titer after 10 minutes at 65°C, and those like the mutant *h* which retain less than 1 percent of the initial titer after 10 minutes at 65°C. A few mutants exhibit intermediate sensitivity, but any that showed a significantly greater sensitivity than wild are designated as sensitive. No segregation of host range and heat sensitivity has been detected to a level of less than 1 percent in crosses between the heat resistant wild-type and the *ht* heat sensitive mutants. The two characters are pleiotropic.

Certain regions of the genetic map (those containing *h*, *ht*4, and *ht*13) frequently produce heat sensitive mutants. The *ht*6 region (which contains 30 percent of all our *ht* mutants) has produced no sensitive mutants. A genetic map prepared from heat sensitive mutants would exhibit much more clustering than one prepared from *ht* mutants.

The heat resistant phenotype permitted BAYLOR and SILVER (1961) to establish that the mutants *h*, *ht*2, *ht*4 and *ht*13 each controlled a part of the phage external protein. After prolonged (20 min) heating of the progeny from cells mixedly infected with mutant and wild type, the sensitive and resistant genotypes were found to be in the same ratio as in the initial unheated sample—thus demonstrating phenotypic mixing, the independent assortment of genotype and phenotype. We have extended these experiments to the new heat sensitive mutants *ht*46 and *ht*49 and have obtained the same result, which shows that these mutants are also determinants of external protein.

Heat treatment of the progeny of mixed bursts also demonstrated that the products of both loci were formed when the wild-type and the mutant alleles were functioning within the same cell. Inactivation curves obtained from time heat inactivation tests showed that both a sensitive and a resistant population of phage particles were formed by the mixedly infected cells (BAYLOR and SILVER 1961). All regions containing heat sensitive mutants have been tested with wild-type, and the progeny of all such crosses, with the possible exception of *ht*46 × ht^+, have acted similarly.

Progeny from crosses within the *ht*13 region, namely *ht*13 × *h*t33, were inactivated similarly to both heat sensitive parents. There was no evidence of wild-type protein resulting from the wild-type alleles of these two closely linked markers. Heat inactivation of progeny from crosses between other heat sensitive mutants gave confusing results which could not clearly be interpreted.

An important observation arose from these experiments. Extensive heat inactivation of second-cycle progeny of both the *ht*4 × *ht*13 and *ht*46 × *ht*49 crosses failed to reduce the *ht* fraction less than 10 percent of the total population. If all *ht* progeny had been heat sensitive like the *ht* parents, less than 1 percent residual *ht* particles would have been expected. Heat inactivation studies of the stocks made from the residual heat resistant *ht* particles showed them to be as resistant as wild type.

It was concluded, therefore, that the heat resistant *ht* particles which resulted from the crosses of the two *ht* heat sensitive parents were the double mutant recombinants—namely *ht*4 *ht*13 and *ht*46 *ht*49. The two double *ht* mutant recombinants are not only heat resistant but also show no additivity in host range phenotype. The additivity of the two heat sensitive gene products and the lack of additivity of the genes in respect to host range strongly suggest that the loci are controlling the same structure if not the same protein.

Temperature sensitivity during development: Temperature sensitive (*ts*) mutants similar to those found in T4 (EDGAR and LIELAUSIS 1964) have been found in T2H after treatment with either HNO_2 or proflavine. These mutants form normal plaques at 25°C, very small plaques at 37°C, and no plaques at

42°C. The *ts* property is associated with sensitivity to temperature during intracellular development and is unrelated to thermal inactivation of mature phage. Several *ts* mutants have been located on the map relative to the *ht* mutants, and are shown in Figure 1. None of the *ht* mutants tested could be described as a *ts* mutant.

The *ts* mutants we isolated affect "late functions". Cells infected with *ts* mutants will produce a full burst of phage if transferred to 32°C after 15 minutes incubation at 42°C. By contrast they produce no phage progeny if they are grown at 32°C for 15 minutes and then transferred to 42°C.

DISCUSSION

Observations reported in this paper establish the circularity of the T2H linkage map. The circularity of the T4 linkage map has been similarly established by STREISINGER, EDGAR, and DENHARDT (1964). Because the DNA of the mature particle of the T-even phages is a linear duplex (RUBENSTEIN, THOMAS, and HERSHEY 1961), the meaning of the circular genetic map requires special theoretical consideration. The circular permutation model of STREISINGER *et al.* (1964), has gained support not only from genetic studies but also from direct physical chemical analysis (THOMAS and RUBENSTEIN 1964).

The *ht* mutant phenotype is expressed by an increased adsorption to the resistant cell B/2 (BAYLOR and SILVER 1961). An increased adsorption could be the result of changes in any structure of the phage which would cause (a) a more specific fit of attachment organs (tail fibers) to the cell wall, (b) more rapid injection to compensate for an inefficient reversible attachment (tail assembly), or (c) more intimate contact between phage and cell surface (net charge). Thus an *ht* mutation may mark any of the structures of the mature phage.

It is reasonable to postulate that the *ht* markers exist in a number of cistrons which control different parts of the morphology of the phage. If a double *ht* mutant or recombinant determined changes in two structures which each increased adsorption, then additivity of different *ht*'s would be expected. The

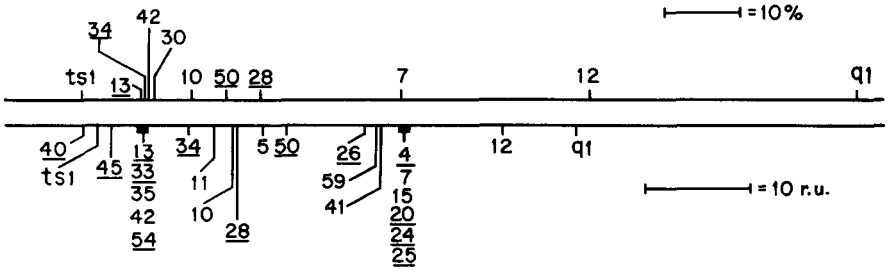

FIGURE 2.—Linkage map of the *ts*1–*q*1 region. The order of the *ht*'s on the lower scale was established by *ht* × *ht* crosses, with *r*1 × *r*13 as an internal control, and distance is in terms of recombination units (r.u.). The order on the upper scale was derived by crossing each *ht* to the end markers *q*1 and *ts*1, and calculating the percent *ts*+ *ht* recombinants among progeny. See Table 3 and RESULTS for explanation. Those *ht*'s which are underlined are heat sensitive; those which are not are heat resistant.

plausibility of this hypothesis is greatly increased by the homologies between the T2H and the T4D linkage map. It has been demonstrated in T4 that different mutants do control different morphological parts (EPSTEIN *et al.*, 1963).

The genes containing the *ht* mutants are not modifiers but control directly the products that are found in the mature phage particle. The evidence for this statement presented first by BAYLOR and SILVER (1961) and extended in this study is: (1) the heat inactivation curves of progeny of mixed bursts of mutant and wild type show that neither allele is dominant but that the products of both alleles are formed; (2) both the heat sensitivity and the adsorption properties exhibit phenotypic mixing. The genetic map prepared with the *ht* mutants is therefore a map of the structural genes of the phage proteins.

The map presented in this study is constructed by adding the recombination distances between closest linked markers. The only correction factor applied was that made possible by the internal $r1 \times r13$ cross which permitted us to correct for unequal parental inputs and progeny of singly infected cells. We have made no attempt to relate linkage data to physical distance. When a suitable mapping function such as proposed by STAHL and STEINBERG (1964) is applied to our data, unmapped areas will increase in length, and clusters of mutants will become condensed, and the proportion of the map occupied by the *ht* markers may become greatly reduced. Nevertheless the *ht* markers will remain scattered. Moreover, there is reason to believe that new *ht* mutants will be found in now unmapped regions of the "late function" portion of the map. It follows that if unmarked genes controlling structural proteins of the mature phage exist, they may be identified among new *ht* mutants.

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SUMMARY

Fifty-two *ht* (extended host range) mutants distributed over two-thirds of the genome, and a variety of other single-step mutants of T2H have been systematically crossed and yield a genetic linkage map that is circular. Heat inactivation of mature phage from mixed bursts indicates that genes with *ht* mutations determine the structural proteins of the mature phage.

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