A MAP OF MOLECULAR DISTANCES BETWEEN MUTATIONS OF BACTERIOPHAGE T4D[1]

J. D. CHILDS[2]

Department of Genetics, University of Washington, Seattle, Washington 98105

Received October 12, 1970

A map of physical distances along the complete T4 DNA molecule has been reported by MOSIG (1968). This map was obtained using T4 phage particles, which contain considerably less DNA than a complete genome (petit particles). Each of these particles contains a continuous and probably random permutation of the T4 DNA molecule. MOSIG estimated the ratio of petit particles which carried a particular am^+ allele and a reference *rI*, *rII*, or *rIII* marker, to those which carried only the am^+ allele. Knowing the length of the DNA contained by the petit particles, the ratio of am^+ to am^+r permits calculation of the distance of the am^+ allele to the *r* mutation. From different crosses, the distances of different am^+ alleles to the *r* reference markers were calculated. Although this method gave consistent results, it has some disadvantages: As the results of different crosses are being compared, the order and distance apart of closely linked markers is difficult to determine; it cannot be used to measure distances within a gene as the petit particle has to carry the whole am^+ gene in order to complement the *am* helper phage; the petit genome must have a uniform known length or inconsistencies result (MOSIG 1966, 1968).

In this paper, a method will be described in which petit particles were used but all the amber mutations to be mapped were carried by one helper phage. The genotypes of individual petit particles were determined by locating each end of their genomes between two amber markers of the helper phage. A map was constructed by assuming that the number of ends between any two markers is proportional to the physical distance separating them. The principal advantage of this method is that all the measurements come from one cross in which the only gene of the petit particle needed to function is *rIIB*. It can therefore be used to measure distances within a gene and gives an unambiguous order of markers even when two are located in the same gene. Furthermore the method is independent of the length of the DNA in the petit particles, which need not be uniform.

MATERIALS AND METHODS

The methods employed are those used by CHASE and DOERMANN (1958) and DOERMANN and PARMA (1967) except where otherwise noted.

Seven T4D strains were used, *E920g* which is a petit mutant, JC10 which carries *rIIB73* and

[1] This investigation was supported by Public Health Service research grant GM13280 from the Institute of General Medical Sciences, National Institutes of Health, Bethesda, Maryland.

[2] Present address: Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada.

amS52 (gene *25*), JC11 which carries *rIIB73* and *amC42* (gene *1*), JC19 which carries *rIIB50* and the following amber mutations (the gene number of each is given in parentheses): *S2(39)*. *N81(41)*, *N82(44)*, *N130(46)*, *lys882*, *C42(1)*, *N135(5)*, *B23(7)*, *N93(11)*, *B20(14)*, *E18(18)*. *B17(23)*, *E355(24)*, *S52(25)*, *N85(48)*, *N54(31)*, *B25(34)*, *B252(35)*, *N52(37)*; JC25, which carries all the markers of JC19 and in addition *A455(34)*; JC53, which carries all the markers of JC25 and *E51(56)*, *N122(42)*, and *B22(43)*; and JC60, which carries all the markers of JC53 and *H17(52)* and *N53(21)*. The genetic order of these markers is given in Figure 3. The *E920g* phage suspension used in crosses with JC19 was an unfractionated lysate containing 1 $r+$ petit phage for every plaque-forming phage. In other crosses, lysates of *E920g* were used with 2–5 $r+$ petit phage for every plaque-forming phage, and also a fraction from a cesium formate density gradient of *E920g* with this ratio increased to about 200 to 1.

Four *Escherichia coli* strains, CR63, CR63(λ*h*), S/6, and B, were used. CR63 is permissive for both *rII* and amber mutants, CR63(λ*h*) restricts *rII* but not amber mutants, and S/6 and B restrict amber but not *rII* mutants. None of these strains restricts whole *E920g* particles.

Genotypes of petit phage: The stepwise procedure for identifying the genotypes of petit phage given below is essentially the same as that used previously (CHILDS 1969).

Step I: *Rescue of petit phage.* Only petit phage, which carried the *rIIB* cistron, were rescued as they had to complement *rIIB50* of the helper phage in a mixed infection in CR63(λ*h*) and give rise to $r+$ whole-phage recombinants able to grow on CR63(λ*h*). The CR63(λ*h*) bacteria was infected with *E920g* phage at a multiplicity of less than 0.01 for crosses with JC19 and less than 0.1 for other crosses, and helper phage (JC19, 25, 53, or 60) at a multiplicity of 2 in the presence of 0.001 M sodium cyanide. After 10 min adsorption at 30°C appropriate dilutions were plated with CR63(λ*h*) to give 20–100 plaques per plate.

Step II: *Determination of genotypes of petit phage.* The plaques on CR63(λ*h*) derived from *E920g* were of two types. The first was from whole *E920g* phage with or without helper phage and the second was from mixed infections of a petit *E920g* phage and helper phage. These two types could be distinguished by testing for ability to grow on B; the former was able to grow on B, whereas the latter could not, because petit genomes did not normally cover all the amber mutations of the helper phage and hence there were no recombinants in the infective centers able to grow on B. It would be possible for a petit particle, carrying more than 81% or more than 86% of the genome, to cover all the amber mutations of the helper if the ends of its genome were to fall between *N130* and *lys882*, or *N54* and *B25*, respectively. However, less than 10% of the petit particles used contained more than 81% of the genome (see RESULTS).

The method of DOERMANN and BOEHNER (1970) was used for testing for ability to grow on B. It consisted of stabbing a plaque with a sterile glass rod and using this to inoculate a small area (5 mm square) on each of 2 plates layered with 2 ml of soft agar seeded with either B or CR63. The latter plate was used as a source of phage for further genotype tests on plaques unable to grow on B. Using a template 50 tests could be made on one plate. Each infective center, unable to grow on B, was tested for $am+$ alleles contributed by the petit phage from which the infective center had been derived, by the replica-plating method of DOERMANN and BOEHNER (1970). Thus the genotype of the petit phage could be deduced; Figure 1 shows the genotype of the

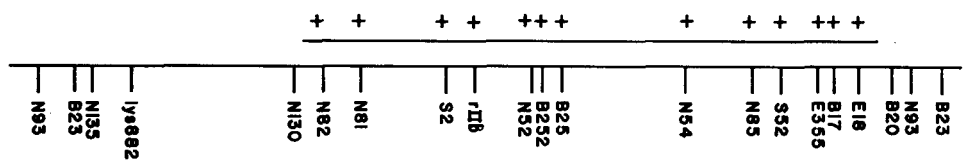

FIGURE 1.—Map of JC19 and a particular petit phage. The upper line indicates the genotype of a particular petit phage and the lower line indicates the genotype of helper phage JC19. The replication procedure tests for the presence of wild-type alleles, which, for an infective center derived from these two phage particles, should indicate a continuous sequence of wild-type alleles from *N82–rIIB–E18*. The clockwise end of the petit genome is therefore between *N82* and *N130* and the counterclockwise end between *E18* and *B20*.

helper phage JC19 and the genotype of a particular petit phage. An infective center derived from these two phage particles should have *am*+ alleles for all the markers *amE18–r50–amN82*, but should lack *am*+ alleles for the other markers *amN130–amB20*.

Revertant *rIIB50* helper phage also gave rise to plaques on CR63(λ*h*) which on testing failed to grow on B. These could readily be distinguished as they did not have any of the wild-type alleles of *E920g* petit phage.

Some petit genomes appeared to be discontinuous and others less than 60% of the physical map long (CHILDS 1969). This was probably due to failure to recover internal or terminal markers, respectively, and data from such phage were not used. These did not account for more than 5% of the phage in crosses with JC19 and a one-step growth lysate of *E920g*, but in later crosses with helper phages JC25, 53, and 60, and other *E920g* lysates this increased to more than 10%.

Most of the markers used gave unambiguous results on replication, although *B17*+ and *E355*+ and to a lesser extent *E18*+, *N53*+, and *lys882*+ frequently gave ambiguous results particularly when present as terminal markers of petit phage. These results were normally not improved by retesting.

The average length of DNA contained by petit particles: The method of MOSIG (1968) was used except that the *rII* mutation was carried by the helper phage rather than the petit phage. *Escherichia coli* B was infected with JC10 (*r73 amS52*) or JC11 (*r73 amC42*) at a multiplicity of 1 and petit particles at a multiplicity of < 0.03. After 10 min adsorption at 30°C, infected bacteria were plated with *E. coli S/6*. The percentage of plaques derived from petit particles, which did not contain *r*+ phage, was determined by testing for inability to grow on CR63(λ*h*) using the method of DOERMANN and BOEHNER (1970). The average length (L) of DNA as a percentage of the genome contained by petit particles was calculated from the equation, $L = \frac{100}{Y + 100}$, where Y is the percentage of plaques which did not contain *r*+ phage. This equation does not include a small correction for the length of the gene in which *amS52* or *amC42* is located.

RESULTS

Mapping the 19 amber mutations of JC19: The genotypes of 3104 petit phage were determined. Each end of each petit genome was assigned to a short region between two of the 19 amber mutations of JC19. As all the petit genomes rescued were *r*+, they each had two ends with respect to the *rII* region, one in a clockwise direction and one in a counterclockwise direction from *rII* on the map of T4. If all petit genomes have a minimum length of 68% of the T4 genome (MOSIG 1968), and if their ends are distributed randomly, then the number of clockwise *or* counterclockwise ends between any two markers should be proportional to their distance apart. For clockwise ends, this will be true up to 68% of the genome from *rIIB* in a clockwise direction. For distances greater than 68%, the frequency of ends between markers will depend on the frequency of petit phage longer than 68%. Likewise for counterclockwise ends this will only be true up to 68% of the genome from *rIIB* in a counterclockwise direction. Thus two maps of two-thirds the genome are obtained, and in a 36% long segment of the genome opposite to *rIIB*, distances can be measured using both clockwise and counterclockwise ends. This region will be called the overlap region. According to the map of MOSIG (1968), it consists of the interval *lys882–S52*. In order to align the two maps any marker in the overlap region could be chosen, as the number of clockwise and counterclockwise ends between it and *rIIB* should equal 100% of the map. Thus

for *B23* (gene 7) there are 2097 clockwise ends and 2241 counterclockwise ends between it and *rIIB*, a total of 4338 or 43.4 per 1 % of the map. This figure could be used to calculate all map distances and *B23* would be 48.3 % of the map from *rIIB* in a clockwise direction. In order to eliminate the bias of selecting a particular marker, the average number of ends between each marker in the overlap region and *rIIB* was used. For the 3104 phage rescued, this was 4362 or 43.6 ends per 1 % of the map. This makes no allowance for the length of the *rIIB* gene, but this is probably not more than 0.6 % of the genome (GOLDBERG 1966). Using this figure the distances of all markers from *rIIB* were calculated (Table 1). This resulted in two maps of two-thirds the genome of T4 (Figure 2). The distances of

TABLE 1

Mapping mutations of JC19

Mutation and gene number	Within each interval		Total from rIIB					This map	Mosig (1968)	Edgar and Wood (1966)	Gene
	Clockwise	Counter-clockwise	Clockwise	Counter-clockwise	Total	Clockwise	Counter-clockwise				
rIIB						0.00		0.0	0.0	0.0	rIIB
	136	0									
amS2(39)			136	3,104	3,240	3.12		3.1	4.9	1.7	39
	395	2									
amN81(41)			531	3,102	3,633	12.17		12.2	14.5	12.8	41
	214	20									
amN82(44)			745	3,082	3,827	17.08		17.1	18.4	19.0	44
	78	10									
amN130(46)			823	3,072	3,895	18.87		18.9	20.6	20.2	46
	791	404									
amlys882(lys)			1,614	2,668	4,282	37.00	61.17	37.9	36.5	35.4	lys
	200	206									
amC42(1)			1,814	2,462	4,276	41.59	56.45	42.6	40.4	40.3	1
	83	71									
amN135(5)			1,897	2,391	4,288	43.49	54.82	44.3	44.5*	46.5	5
	200	150									
amB23(7)			2,097	2,241	4,338	48.08	51.38	48.4	48.2*	49.5	7
	140	133									
amN93(11)			2,237	2,108	4,345	51.29	48.33	51.5	52.7	53.1	11
	132	105									
amB20(14)			2,369	2,003	4,372	54.31	45.92	54.2	56.4	54.9	14
	149	149									
amE18(18)			2,518	1,854	4,372	57.73	42.51	57.6	58.1	57.7	18
	171	65									
amB17(23)			2,689	1,789	4,478	61.65	41.02	60.3	60.8	60.9	23
	76	102									
amE355(24)			2,765	1,687	4,452	63.39	38.68	62.4	61.3	62.8	24
	181	220									
amS52(25)			2,946	1,467	4,413	67.54	33.63	67.0	66.0*	68.0	25
	89	111									
amN85(48)			3,035	1,356	4,391		31.09	68.9	68.9*	72.0	48
	45	317									
amN54(31)			3,080	1,039	4,119		23.82	76.2	76.0	78.3	31
	20	602									
amB25(34)			3,100	437	3,537		10.02	90.0	91.4	+	34
	4	106									
amB252(35)			3,104	331	3,435		7.59	92.4	92.5	+	35
	0	63									
amN52(37)			3,104	268	3,372		6.14	93.9	94.5	96.0	37
	0	268									
rIIB							0.00	100.0	100.0	100.0	rIIB
	3,104	3,104									

Mean number of ends between rIIB and each marker in the interval lys882-S52 = 4,362

The mean number of clockwise and counterclockwise ends of petit genomes between each marker from *lys882–S52* and *rIIB* was used to calculate the distances of all markers to *rIIB*. This resulted in two maps of two-thirds the genome of T4 and an average map of the whole genome. Distances calculated from the maps of MOSIG (1968) and EDGAR and WOOD (1966) are given for comparison.

* Interpolation based on map of EDGAR and WOOD (1966).

† Distance gene *31*–gene *37* reduced from 26.9% to 17.7% to coincide with the distance on the physical map. Other distances were increased proportionately.

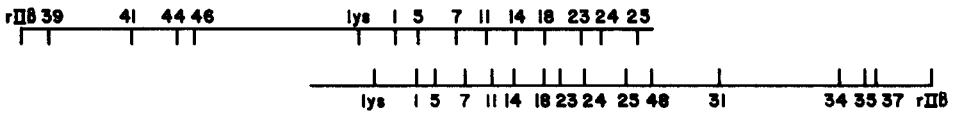

FIGURE 2.—Maps of T4 obtained using clockwise and counterclockwise ends of petit genomes. The upper line is the map obtained using clockwise ends of petit genomes and the lower line the map obtained using counterclockwise ends. For the mutation mapped in each gene see MATERIALS AND METHODS.

the markers in the overlap region were averaged from the two maps (Table 1 and Figure 3). As expected, the region *lys882–S52* was within the overlap region and also possibly *S52–N85*, judged by the similar numbers of clockwise and counterclockwise ends in this region (see below).

Two criteria may be used to judge the accuracy of the map. First, in the overlap region the numbers of clockwise ends should equal the number of counterclockwise ends within each interval. In 6 of the 9 intervals, there was no significant difference in the number of ends ($P > 0.05$) and in 2 intervals there was a small difference which was significant ($P < 0.05$). In one interval between *E18* and *B17* there was a considerable difference, significant with $P < 0.001$ (171

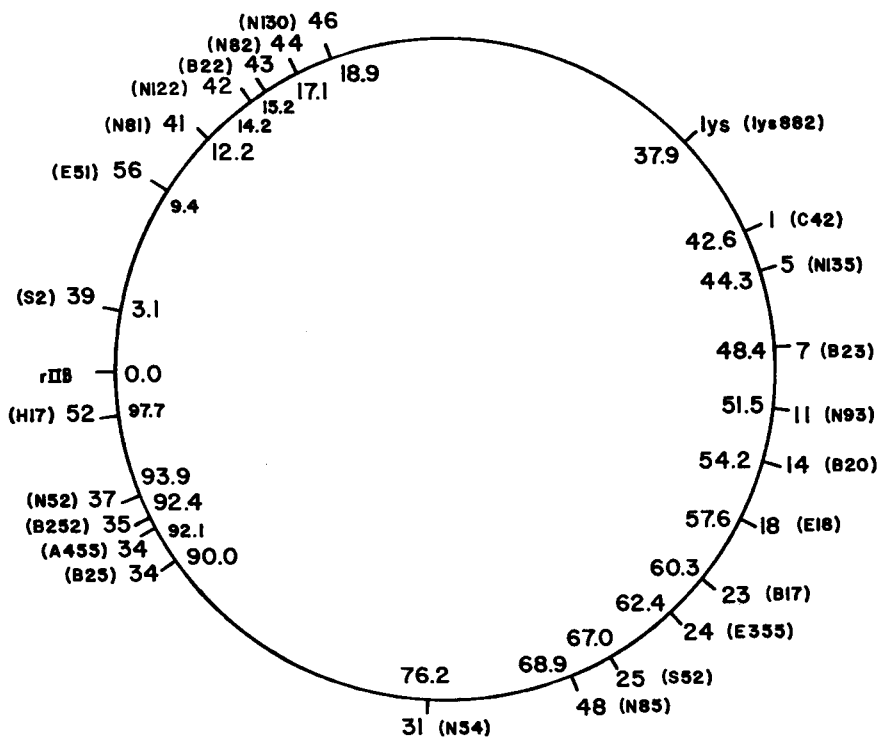

FIGURE 3.—Map of physical distances between mutations of T4. The mutant numbers are given in parentheses next to the number of the gene in which they are located. The numbers inside the circle are the percentages of the physical map from *rIIB*, those in larger print corresponding to the mutations of JC19.

TABLE 2

Comparison of clockwise and counterclockwise ends between markers in the overlap region (lys882–S52)

Mutation	Number of ends in each interval		χ^2	P (df=1)
	Clockwise	Counterclockwise		
lys882				
	200	206	0.08	>0.90
C42				
	83	71	0.94	0.30–0.50
N135				
	200	150	7.14	0.001–0.01
B23				
	140	133	0.18	0.80–0.90
N93				
	132	105	3.08	0.05–0.10
B20				
	149	149	0	>0.90
E18				
	171	65	47.62	<0.001
B17				
	76	102	3.98	0.02–0.05
E355				
	181	220	3.79	0.05–0.10
S52				
Total				
E18–S52	428	387	2.06	0.10–0.20
lys882–S52	1,332	1,201	6.78	0.001–0.01

Clockwise and counterclockwise ends of petit genomes should be equally frequent within each interval between *lys882* and *S52*, provided that petit particles contain a random segment of not less than 67% of the T4 DNA molecule, and that terminal markers are recovered with equal efficiency.

clockwise ends and only 67 counterclockwise ends). The reason for this may have been difficulty in recognizing *B17*$^+$ and *E355*$^+$ when present at the ends of a petit genomes. Thus, within the first two of the intervals *E18–B17–E355–S52*, the differences in the number of ends are significant, but the difference between the total numbers of ends between *E18* and *S52* is not (Table 2). There were also approximately 10% less counterclockwise than clockwise ends in the overlap region *lys882–S52* ($P < 0.01$), but the least difference required for significance at $P = 0.05$ was only slightly less than this (7.5%).

Second, for a marker in the overlap region the total number of clockwise and counterclockwise ends between it and *rIIB* should equal 4362. The range found was 4276–4478, an error of not more than ± 2.7%.

Thus, although there appeared to be small deviations from expected values, probably because of nonrandom loss of terminal markers, these were not considered large enough to invalidate the calculations of map distances, except possibly for *B17* and *E355*.

Mapping mutations E51, N122, B22, N53, A455, *and* H17: Having obtained a map of T4 based on the 19 amber mutations of JC19, further crosses were carried

out using different helper phages. Instead of analyzing the genotypes of all the infective centers from the crosses, only those plaques derived from petit genomes with ends in particular regions were selected for analysis. For example, in order to map *E51*, *B22*, and *N122*, helper phages JC53 and JC60 were used and infective centers replicated first to *N82 or N130*. Those infective centers which did not carry *N82*$^+$ or *N130*$^+$ had been derived from petit genomes with clockwise ends between *rIIB* and *N82* or *N130*. All of these were replicated to all the markers carried by JC53 or JC60 in order to locate their ends. Selection was also made for ends between *N81* and *N82* to obtain more data within this interval. From the ratio of ends between markers, the distance of *E51* from *S2* and *N81* and the distances of *N122* and *B22* from *N81* and *N82* were calculated (Table 3). For example, between *S2* and *N81* there were 210 ends, of which 69.5 % were between *S2* and *E51* and 30.5 % were between *E51* and *N81*. As the distance from *S2* to *N81* is 9.1 % of the map, the distance *S2* to *E51* is 6.3 % and *E51* to *N81* is 2.8 % of the map (Tables 1 and 3). Similarly, *A455* and *H17* were mapped by selection of ends between *rIIB* and *B25* (Table 3).

TABLE 3

Mapping mutations E51(56), N122(42), B22(43), N53(21), A455(34), *and* H17(52)

Genetic interval	Number of ends in each interval		Percentage of ends between mutations of JC19	Interval size, percent of map	Percentage of map from *rIIB* from			
	Clockwise	Counter-clockwise			This map	Mosig (1968)	Edgar and Wood (1966) modified†	Gene (mutation)
S2–E51	146	. .	69.5	6.3	3.1	4.9	1.7	*39 (S2)*
E51–N81	64	. .	30.5	2.8	9.4	12.1	9.1	*56 (E51)*
S2–N81	210	. .	100.0	9.1‡	. .	. . .	. . .	. .
N81–N122	68	. .	41.7	2.0	12.2	14.5	12.8	*41 (N81)*
N122–B22	33	. .	20.2	1.0	14.2	15.6*	14.7	*42 (N122)*
B22–N82	62	. .	38.0	1.9	15.2	16.2	17.5	*43 (B22)*
N81–N82	163	. .	100.0	4.9‡	17.1	18.4	19.0	*44 (N82)*
E18–N53	27	12	see text					
N53–B17	17	3	see text					
B25–A455	. .	128	85.3	2.1	90.0	91.4	†	*34 (B25)*
A455–B252	. .	22	14.7	0.4	92.1	91.4	†	*34 (A455)*
B25–B252	. .	150	100.0	2.4‡	92.4	92.5	†	*35 (B252)*
N52–H17	. .	85	62.5	3.9	93.9	94.5	96.0	*37 (N52)*
H17–rIIB	. .	51	37.5	2.3	97.7	97.6	97.3	*52 (H17)*
N52–rIIB	. .	136	100.0	6.1‡				

Ends were selected between mutations already mapped using helper phages JC25, 53, and 60, which carry additional mutations. The relative positions of these mutations were calculated from the proportion of ends between these and the nearest mutations of JC19. Numbers within one pair of lines in the table, but not necessarily within two pairs of lines, come from the same experiment. It is therefore not meaningful to compare, for example, the number of ends between *S2* and *N81* with the number between *N81* and *N82* since different totals were tested in the two experiments. Distances calculated from the maps of Mosig (1968) and Edgar and Wood (1966) are given for comparison.

* Interpolation based on map of Edgar and Wood (1966).
† For method of calculation, see legend to Table 1.
‡ Interval mapped previously, see Table 1.

It had been noticed that there was a considerable difference in the numbers of clockwise and counterclockwise ends between *E18* and *B17*. To investigate this further, selection was made for ends in this region using helper phage JC60, which carries *N53* located between these two markers. Within both of the intervals *E18–N53* and *N53–B17* there were more clockwise than counterclockwise ends. For this reason no attempt was made to obtain more data or to map *N53*, although it did appear to be closer to *B17* than *E18*.

Most of the data used to map *E51*, *N122*, *B22*, *A455*, and *H17* came from crosses using a sample of *E920g* from a cesium formate gradient, in which the frequency of discontinuous and short genomes was higher than in crosses used to map the mutations of JC19. The two sets of data were therefore tested for homogeneity (Table 4); the ends between *rIIB* and *B25* were homogeneous ($P = 0.7–0.8$), but the ends between *rIIB* and *N82* were not ($P < 0.01$) because there were fewer than the expected number of ends between *rIIB* and *S2*. A part of the reason for this may have been that in selecting for infective centers lacking *N82*$^+$ or *N130*$^+$, those derived from long petit genomes with a clockwise end between *rIIB* and *S2* and a counterclockwise end between *S2* and *N82* or *N130* were eliminated. This should have a negligible influence on the mapping of *E51* and none on *N122* and *B22*.

Distribution of lengths of DNA in petit E920g *particles:* It is apparent from the mapping data that most of the petit particles contained about 67–70% of the

TABLE 4

Homogeneity tests of data obtained from crosses with E920g from a mass lysate and from a cesium formate gradient

Mutation	Clockwise ends in each interval		χ^2	P (df=2)
	Mass lysate	Gradient		
rIIB				
	136	40		
S2				
	395	198	7.27	0.02–0.05
N81				
	214	100		
N82				
	Counterclockwise ends			
B25				
	106	47		
B252				
	63	27	0.54	0.7–0.8
N52				
	268	103		
rIIB				

The data given in Table 3 came from crosses using a sample of *E920g* from a cesium formate density gradient, in which the frequency of aberrant phage was higher than in crosses used to map JC19. To test the validity of the data both sets were compared, using two independent chi-squared tests, one for clockwise ends between *rIIB* and *N82* and the second for counterclockwise ends between *rIIB* and *B25*. There were fewer than expected ends between *rIIB* and *S2* but this should have a negligible influence on the mapping of the mutations given in Table 3 (see text).

genome. This is evident from the equal numbers of clockwise and counterclockwise ends, both between markers in the overlap region and as far as *N85* in a clockwise direction, a distance of 68.9% from *rIIB*. It is also evident from the reduced frequency of clockwise ends beyond *N85*; there were only 45 clockwise ends between *N85* and *N54* compared to 317 counterclockwise ends, and only 24 clockwise ends beyond *N54*. If it is assumed that all petit particles contain only 68%, 78%, or 91% of the T4 genome (Mosig 1968), then an estimate of the ratios of these three classes can be obtained from the frequency of clockwise ends between *B25* and *N54*, *N54* and *N85*, and *S52* and *rIIB*. Clockwise ends of "91% genomes" should be distributed randomly between *B25* and *N54*; clockwise ends of both "91%" and "78% genomes" should be distributed randomly between *N54* and *N85*; and clockwise ends of all three classes should be distributed randomly between *S52* and *rIIB*. The frequency of ends in these three regions was 1.5, 6.2, and 43.6 per 1 % of the physical map, respectively. Thus if petit particles contain only 91%, 78%, or 68% of the genome, the relative frequencies of these are 3%, 11%, and 86%, respectively. However, if some or all of the "68 %" class are in fact longer than 69% of the genome, then some of them will end between *N85* and *N54* thus increasing the apparent numbers of "78 %" particles.

It should be possible to confirm the relative sizes and frequencies of petit particles from a classification of each genome according to the locations of both of its ends (Table 5). However, most of the petit genomes have either or both ends in long intervals (5 % or more of the map) and the distribution of short intervals is such that if only petit genomes with both ends in short intervals are considered, the frequency of certain sizes would be exaggerated. An alternative is to examine all the petit genomes which have one end in a particular short interval. For example, petit particles which contain 60–80% of the T4 genome with one end between *N82* and *N130*, or *B25* and *B252*, or *B252* and *N52*, will have another end in one of the intervals between *lys882* and *N85*, which are 2–5 % of the genome long. Petit particles which contain more than 80% of the genome with a clockwise end in one of the intervals between *lys882* and *B23* or a counterclockwise end in one of the intervals between *N85* and *E18* will have another end between *lys882* and *N85*. Thus it is possible to examine separately two classes of petit particles, those which contain 60–80 % of the genome and those which contain more than 80 % of the genome.

Of the 247 genomes with one end in either of the intervals *N82–N130*, *B25–B252*, or *B252–N52*, an average of 59.9% had another end in a single short interval. 80.5 % had another end in either of two contiguous intervals and 86.6 % had another end in one of three contiguous intervals (Tables 5 and 6). This result suggests that more than 80 % of the petit genomes are of uniform size. The average distance of the map covered by these genomes, which was calculated from the midpoints of their terminal intervals, was 69.3% (69.5% from the map of Mosig 1968). This is likely to be an underestimate of the actual size due to loss of terminal markers. Although this appeared to be slight (see next section), most of the genomes with one end between *B25* and *B252* had another end between *E18* and *S52*, where loss of terminal markers *B17*$^+$ and *E355*$^+$ appeared to be

TABLE 5

Genotypes of petit phage used to map mutations of JC19

Clockwise terminal markers	Counterclockwise terminal markers (all *am*+ except *rIIB*+)																			
	rIIB	*N52*	*B252*	*B25*	*N54*	*N85*	*S52*	*E355*	*B17*	*E18*	*B20*	*N93*	*B23*	*N135*	*C42*	*lys*	*N130*	*N82*	*N81*	*S2*	Total
rIIB	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	121	3	9	2	..	136
S2	..	..	..	..	..	..	..	..	..	..	3	4	4	5	106	255	7	11	..	..	395
N81	..	..	..	..	..	..	..	..	..	2	2	8	44	53	79	26	..	..	..	..	214
N82	..	..	..	..	..	..	..	..	..	3	1	11	56	2	3	2	..	..	..	..	78
N130	..	..	..	9	31	29	178	84	55	136	97	103	42	9	18	..	..	..	..	..	791
lys882	..	..	..	3	86	67	20	7	3	1	2	7	3	1	..	..	..	..	..	..	200
C42	..	..	..	2	71	2	3	..	1	3	..	..	1	..	..	..	..	..	..	..	83
N135	..	..	..	97	84	6	2	6	4	1	..	..	..	..	..	..	..	..	..	..	200
B23	..	..	..	121	9	2	2	3	..	3	..	..	..	..	..	..	..	..	..	..	140
N93	2	1	2	110	9	1	6	..	1	..	..	..	..	..	..	..	..	..	..	..	132
B20	6	1	2	127	5	4	2	1	1	..	..	..	..	..	..	..	..	..	..	..	149
E18	7	11	66	77	6	..	3	1	..	..	..	..	..	..	..	..	..	..	..	..	171
B17	12	26	24	6	4	..	4	..	..	..	..	..	..	..	..	..	..	..	..	..	76
E355	136	16	2	16	11	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	181
S52	69	2	2	15	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	89
N85	19	2	5	19	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	45
N54	16	1	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	20
B25	1	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4
B252	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0
Total	268	63	106	602	317	111	220	102	65	149	105	133	150	71	206	404	10	20	2	0	3,104

All petit phage rescued were *rIIB*+ from the same lysate of *E920g*. Each figure in the table is the total number of phage genomes with identical terminal markers.

TABLE 6

Distribution of ends of petit genomes with one end between N82 *and* N130, B25 *and* B252, *or* B252 *and* N52

Number of ends	First end	Percent distribution of second end					Weighted mean distance covered by majority classes	
		Minority classes		Majority classes				
				Contiguous intervals*			Percent of map of CHILDS MOSIG (1968)	
		Short	Long	1	2	3		
78	*N82 –N130*	5.1	6.4	2.6	71.8	14.1	71.1	72.6
106	*B25 –B252*	1.8	11.3	1.8	62.3	22.6	68.3	67.9
63	*B252–N52*	3.2	12.7	17.5	41.3	25.4	68.7	68.1
Weighted mean		3.2	10.1	6.1	59.9	20.6	69.3	69.5

All petit phage containing 60–80% of the T4 genome with one end between *N82* and *N130*, *B25* and *B252*, or *B252* and *N52* have a second end between *lys882* and *N85*. Markers in the latter region are not more than 5% of the map apart (average 3.1%). The majority of the "second ends" were clustered in two or three contiguous intervals, with a minority of the ends in several intervals corresponding to longer and shorter genomes. The contiguous intervals have been numbered such that interval 2 is the interval with the most ends, and 1 and 3 are the adjacent intervals with more ends in 3 than 1.

* The contiguous intervals are:

First end	1	2	3
N82 – N130	*C42 – N135*	*– B23*	*– N93*
B25 – B252	*B20 – E18*	*– B17*	*– E355*
B252 – N52	*E18 – B17*	*– E355*	*– S52*

frequent (see *mapping* section). The average distance of the map covered by these genomes was 68.5 % compared to 71.1 % for those with one end between *N82* and *N130* (Table 6). The remainder of the genomes, 3.2% covering shorter distances and 10.1% longer distances, did not appear to have ends clustered in any particular intervals and therefore no class corresponding to 78% of the map could be distinguished.

The genomes longer than 80% of the map with a clockwise end in one of the intervals between *lys882* and *B23* or counterclockwise end between *N85* and *E18* also did not appear to have ends clustered in any particular intervals. However, of the 482 genomes in the former class and 498 genomes in the latter class, 7.3% and 9.4%, respectively, covered more than 80% of the map, including 4.8% and 5.0%, respectively, which covered 85.0–90.3 % of the map.

In summary, these results suggest that in the one-step growth lysate of *E920g* used for these experiments, 80–87% of the petit particles contain 69–71% of the T4 genome, that 10% contain more than this including 5% which contain 85–90% of the genome, and that 3–10% contain less than 70% of the genome. Loss of terminal markers of petit genomes may account for all of those less than 70% of the map, and loss of markers from whole *E920g* could account for some of the genomes longer than 90% of the map. In some cases a cell infected with two "70%" phages would have the appearance of having been infected with one longer phage. However, with the multiplicity of infection used (< 0.01) this would be less than 1% of the infected cells.

Average length of DNA contained by petit particles: Loss of terminal markers

could be estimated by a comparison of the average length of DNA contained by a sample of petit particles, calculated from the mapping data with that calculated using the genetic method of MOSIG (1968). The latter was shown to yield results consistent with those obtained with DNA, extracted from petit particles, measured by sedimentation through sucrose gradients. The average length of DNA contained by the petit particles can be obtained from the mapping data by a method analogous to that used by MOSIG. That is, from the percentage of r^+ petit particles which do not carry a particular am^+ marker in the overlap region. This can be calculated for each of the markers in the overlap region and the average substituted for Y in the equation, $L = \frac{100}{Y + 100}$ where L is the average length of DNA contained by the petit particles, as a percentage of the complete T4 genome (see METHODS).

The average length of DNA contained by the petit particles of the mass lysate, calculated from the mapping data, was 71.7% of the genome (Table 7). This is consistent with the results obtained from the analysis of individual classes of particles (see previous section). MOSIG's method was not used for the mass lysate, because the presence of as many whole particles as petit particles would have involved too large a correction factor. For the density gradient sample, the average length of DNA measured by MOSIG's method was 66.8% of the genome. This was 3% less than that calculated from the mapping data for the same gradient sample, and 5% less after correcting the mapping data for genomes less than 60% of the map and for discontinuous sequences. Terminal markers, therefore, appear to be recovered efficiently and loss of all markers appears to be slightly less with this method than with the method of MOSIG.

DISCUSSION

The map obtained by the method described in this paper appears to be reliable, judging from the similarity of the two fractional maps (Figure 2) and by the

TABLE 7

Average length of DNA contained by petit particles

Source of phage	Method of calculation: From mapping data, Mass lysate	From mapping data, Gradient*	MOSIG (1968), Gradient
Total petit particles	221	141 (169)	2,030
Total markers tested	1,989	1,269 (1,521)	2,030
Percentage of markers *am* (Y)	39.5	38.8 (43.2)	....
or			
Percentage of markers *r73* (Y)	..	... .	49.7
Average length of DNA (L)	71.7	72.0 (69.8)	66.8

The average length (L) of DNA contained by petit particles was calculated from the equation $L = \frac{100}{Y + 100}$ (see METHODS). The value Y was the percentage of petit particles which did not have a particular marker. For the mapping data this was the average of 9 am^+ markers located in the overlap region and for MOSIG's method this was $r73^+$.

* Figures in parentheses have not been corrected for 10 genomes less than 60% of the map and 18 discontinuous sequences.

similarity of this map and that of MOSIG (1968). Differences in distance between markers on both maps did not exceed 2.7 % of the genome of T4, and for 12 markers the difference was less than 1% (Tables 1 and 3). There are two principal differences between the maps: First, in this map genes 5 and 7 are in the same order as in the genetic map of EDGAR and WOOD (1966); the second difference is that with this method mutations rather than genes are mapped. As a result, mutations within the same gene can be mapped and a maximum estimate of the distance between genes can be obtained. Thus the mutations *B25* and *A455* within gene *34* are further apart than *A455* (gene *34*) and *B252* (gene *35*). The minimum size of gene *34* is thus 2.1% and the maximum distance between genes *34* and *35* is 0.4 % of the genome (Table 3). The physical distance between *B25* and *A455* is less than one-half of the distance on the recombination map of DOERMANN and PARMA (1967), but this nevertheless indicates that gene *34* is at least twice the size of the average T4 gene.

It may be noted that the statistical error in the measurement of short distances is much less than in the method used by MOSIG (1968), as the number of ends between adjacent markers is estimated directly rather than by difference. A more serious potential error is the variable loss of terminal markers. This could be caused by difficulty in recognizing particular markers in the replication procedure, or by local variation in recombination frequency or marker rescue efficiency. Thus, WOMACK (1965) found that markers from a UV-irradiated phage differed in their probability of being rescued by as much as a factor of eight. Markers with the highest probability of being rescued were located in and near genes *2*, *25*, *34*, and *43*. A high recombination frequency within gene *34* could increase the apparent size of this gene. Despite this, the sizes of genes *35* and *34*, and the distances between genes *34*, *35*, and *37* are much smaller than on the recombination map. If allowance is made for this difference, then all the distances on the map of EDGAR and WOOD (1966) are similar to those on this map, including *rIIB*–gene *56*, which is slightly longer on the map of MOSIG (1968); (Tables 1 and 3).

In order to obtain the most reliable estimate of the distance between closely linked markers, it would be necessary to use both clockwise and counterclockwise ends. This would detect nonrandom loss of particular terminal markers as appeared to be the case with *E335*$^+$ and *B17*$^+$. Using a purified preparation of petit particles each containing 90% of the genome, this should be possible for all genes from *41–34* clockwise. Alternatively, it should be possible to rescue and identify random petit particles, not just those which carry *rIIB*$^+$, by using CR63 instead of CR63(λh) and by increasing the multiplicity of infection of the petit phage. In this way all genes could be mapped using the ends of petit genomes oriented in opposite directions.

The *E920g* lysate used to map the markers of JC19 appeared to be a mixture of sizes of petit particles. The majority, more than 80% of the total, appeared to contain a uniform length of 69–71% of the T4 genome. This is slightly longer than the 67.7 % found by MOSIG (1966, 1968) for the smallest size of petit particles isolated from wild-type T4. Although loss of terminal markers appeared to be small, some loss is inevitable, and therefore the estimate for the size of DNA

contained by petit particles of the *E920g* mutant is probably a minimum one. Approximately 10% of the particles contained more than 71% of the T4 genome, and although 50% of these contained 85–90% of the genome, and may correspond to the "91%" particles of MOSIG (1968), the remainder were of variable size. PARMA (1969) did not find any petit particles from an *E920g* lysate which contained as much as 91% of the genome, but this may have been the result of using a selected fraction from a density gradient. The proportions of petit particles of different sizes may vary in different lysates of *E920g*, although there appeared to be no detectable variation between single bursts, despite considerable variation in the proportions of whole and petit particles (CHILDS 1969).

I wish to thank Dr. A. H. DOERMANN for many valuable discussions and for reading and criticizing the manuscript, and Mrs. EMIKO CHILDS for technical assistance.

SUMMARY

Petit phage particles of T4 appear to contain a random circular permutation of the T4 DNA molecule. Identification of the ends of a random sample of these molecules, which included *rIIB*$^+$, was used to map 24 amber mutations of T4 with respect to *rIIB*. The gene order was the same as for the recombination map, and distances were similar except for genes *34* and *35* which were much longer on the recombinational map.—The lysate of the petit mutant *E920g* contained a mixture of sizes of petit particles: 80–87% of the total contained 69–71% of the genome; 10% contained more than this, including 5% which contained 85–90% of the genome; and 3–10% contained less than 70% of the genome.

LITERATURE CITED

CHASE, M. and A. H. DOERMANN, 1958 High negative interference over short segments of the genetic structure of bacteriophage T4. Genetics **43**: 332–353.

CHILDS, J. D., 1969 Permuted partial phage within single bursts of *Escherichia coli* infected with a T4D mutant. Genetics **63**: 239–246.

DOERMANN, A. H. and L. BOEHNER, 1970 The identification of complex genotypes in bacteriophage T4. I: Methods. Genetics **66**: 417–428.

DOERMANN, A. H. and D. H. PARMA, 1967 Recombination in bacteriophage T4. J. Cellular Physiol. **70** (Suppl. 1): 147–164.

EDGAR, R. S. and W. B. WOOD, 1966 Morphogenesis of bacteriophage T4 in extracts of mutant-infected cells. Proc. Natl. Acad. Sci. U.S. **55**: 498–505.

GOLDBERG, E. B., 1966 The amount of DNA between genetic markers in phage T4. Proc. Natl. Acad. Sci. U.S. **56**: 1457–1463.

MOSIG, G., 1966 Distances separating genetic markers in T4 DNA. Proc. Natl. Acad. Sci. U.S. **56**: 1177–1183. ——, 1968 A map of distances along the DNA molecule of phage T4. Genetics **59**: 137–151.

PARMA, D. H., 1969 The structure of genomes of individual petit particles of the bacteriophage T4D mutant *E920/96/41*. Genetics **63**: 247–261.

WOMACK, F. C., 1965 Cross-reactivation differences in bacteriophage T4D. Virology **26**: 758–760.