STUDIES OF BACTERIOPHAGE T4

TAIL FIBERS AND TAIL FIBER GENES

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Abstract

The distal half of the bacteriophage T4 tail fiber interacts with the surface of the bacterium during adsorption. The largest polypeptide in this half fiber is the product of gene 37 (P37). During assembly of the tail fiber, P37 interacts with the product of gene 38 (P38). These two gene products are incompatible with the corresponding gene products from the related phage T2. T2 P37 does not interact with T4 P38 and T2 P38 does not interact with T4 P37. Crosses between T2 and T4 phages mutant in genes 37 and 38 have shown that the carboxyl end of P37 interacts with P38 and with the bacterial surface. In the corresponding region of gene 37 and in gene 38 there is no recombination between T2 and T4. In the rest of gene 37 there are two small regions with relatively high recombination and a region of low recombination.

When T2/T4 heteroduplex DNA molecules are examined in the electron microscope four nonhomologous loops appear in the region of genes 37 and 38. Heteroduplexes between hybrid phages which have part of gene 37 from T4 and part from T2 have roughly located gene 37 mutations in the heteroduplex pattern. For a more precise location of the mutations a physical map of gene 37 was constructed by determining the molecular weights of amber polypeptide fragments on polyacrylamide gels in the presence of sodium dodecyl sulfate. When the physical and heteroduplex maps are aligned, the regions of low recombination

correspond to regions of nonhomology between T2 and T4. Regions with relatively high recombination are homologous.

The molecular weight of T2 P37 is about 13,000 greater than that of T4 P37. Analysis of hybrid phage has shown that this molecular weight difference is all at the carboxyl end of P37.

An antiserum has been prepared which is specific for the distal half fiber of T4. Tests of the ability of gene 37 hybrids to block this antiserum show that there are at least 4 subclasses of antigen specified by different parts of P37.

Observations in the electron microscope of the tailfiber - antibody complexes formed by the gene 37 hybrids and the specific antiserum have shown that P37 is oriented linearly in the distal half fiber with its N-terminus near the joint between the two half fibers and its C-terminus near the tip of the fiber. These observations lead to a simple model for the structure of the distal half fiber.

The high recombination in T4 gene 34 was also investigated. A comparison of genetic and physical maps of gene 34 showed that there is a gradient of increasing recombination near one end of the gene.

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General Introduction

Interactions between protein molecules and complex carbohydrates on the surface of cells are important in a variety of biological processes. Interaction of humoral or cellular antibodies with cellular carbohydrate antigens, cell-cell recognition and sorting during development, and attachment of viruses to the surface of host cells are all examples of this type of interaction. The cellular receptors for a number of viruses have been intensively studied and in several cases the composition and sequence of monomers in the polysaccharide portion of the receptor have been determined. In bacterial systems, cells with altered bacteriophage receptors have been used to determine which part of the receptor molecule is necessary for the attachment of a particular phage. Very clear requirements have emerged for a number of phages (Rapin and Kalckar, 1971).

However, in none of these cases has the second component of the system, that part of the phage which interacts with the surface of the bacterium, been investigated. In fact, most of the work has been done with phages which are quite poorly characterized by criteria other than their adsorption specificity.

These considerations led me to study adsorption of bacteriophage T4, a coliphage whose adsorption apparatus has been intensively investigated. Most of this thesis is a study of the structure of the tail fibers of T4 which interact with the bacterial surface during adsorption. Some experiments aimed at understanding the bacterial

receptor for these fibers are described in the general discussion.

T4 tail fibers were chosen for study for a number of reasons. During adsorption the tips of these tail fibers attach specifically to the lipopolysaccharide of the bacterial cell wall (Simon and Anderson, 1967; Wilson, Luftig and Wood, 1970). T4 is very easy to manipulate genetically. There are a large number of mutants available, and functional and recombinational analysis of them is rapid and precise. Genes specifying all of the major polypeptides of the tail fiber have been identified as well as two genes which are necessary for tail fiber assembly but do not contribute proteins to the structure (King and Laemmli, 1972; Ward and Dickson, 1972; Eiserling and Dickson, 1972). Most of the pathway of tail fiber assembly has been worked out (Edgar and Lielausis, 1965; King and Wood, 1969).

Parts I and II of this thesis are concerned with the structure of the distal half of the T4 tail fiber, the structure of gene 37, which codes for the major polypeptide of the distal half fiber, and of gene 38, the product of which interacts with the gene 37 product during tail fiber assembly. As will be seen in part I the gene 37 product is the protein which interacts with the bacterial receptor.

Part I is a comparison of genes 37 and 38 between T4 and the closely related phage T2, which adsorbs to different bacterial receptors than T4. The parts of these genes which have become non-homologous during divergence of T4 and T2, and the part of the gene 37 product which interacts with the bacterium and with the gene 38 product are identified.

Part II describes the distribution of antigens along the tail fiber and the conformation of the gene 37 product in the assembled fiber.

Section III describes an investigation unrelated to the structure of the tail fiber. It is shown that the large genetic size of T4 gene 34 results from a gradient of increasing recombination near the end of gene 34.

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STRUCTURE OF BACTERIOPHAGE T4 GENES 37 and 38

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1. Introduction

Bacteriophages T2 and T4 are so closely related that a gene product missing from one of the phages due to an amber mutation can usually be replaced by the corresponding gene product from the other phage (Russell, 1967). The only two exceptions are the products of genes 37 and 38 (P37 and P38). These interact during assembly of the distal half of the phage tail fiber (King & Wood, 1969), P37 being incorporated as the major polypeptide of this half fiber (Ward et al., 1970). P38, while necessary for fiber formation, is not incorporated into the assembled structure (King & Laemmli, 1972; Eiserling & Dickson, 1972). During adsorption the distal half fiber interacts with specific receptors on the surface of the bacterium (Wilson, Luftig & Wood, 1970). The specificity of this interaction is at least partially controlled by P37, since host range mutations of T4 map in gene 37 (Beckendorf, in preparation). P37 and P38 cannot be exchanged by T2 and T4 because P37 from T4 ($P37^4$) cannot interact with P38 from T2 ($P38^2$) and $P37^2$ cannot interact with $P38^4$ (Russell, 1967). The T2 and T4 gene products must also differ functionally, since the two phages attach to very different receptors on the bacterial surface (Jesaitis & Goebel, 1953). Despite these differences there is a low level of am^+ recombinants in crosses of the type T2 am37 x T4 am38 or T4 am37 x T2 am38 (Russell, 1967). These results suggest that, despite the incompatibility of the finished T2 and T4 gene products, there are at least some regions of the two genes which are mutually compatible.

To determine which regions are compatible and which are incompatible, we have carried out intertype crosses between T2 and T4 mutants defective in genes 37 and 38. Analysis of these crosses and of the T2:T4 hybrid phage produced has allowed us to locate the host range (h) determinant, the site of P37 interaction with P38, and the regions of these genes which are not homologous between T2 and T4.

2. Materials and Methods

(a) Phage and bacterial strains

Phage strains derived from the wild type T4D were obtained from the collection of R. S. Edgar and have been described elsewhere (Epstein et al., 1963; Edgar & Lielausis, 1965; Wilson & Kells, 1972; Bernstein, Edgar & Denhardt, 1965). T4B rH23 is an rII deletion mutant of T4B which is missing both the rIIA and rIIB cistrons (Benzer, 1959). Most of the phage strains derived from wild type T2L were obtained from the collection of R. L. Russell and have also been previously described (Russell, 1967). T2 rH23 is a recombinant between T2L and T4B rH23 which was selected to retain T2 host range, and the rII deletion. It is homologous with T2 throughout the tail fiber region (Kim & Davidson, 1972). T2:T4 hybrid phage made during this study are described in Table 1.

Escherichia coli strain CR63 was used as permissive host for T4 amber mutants, strain CR63$r_6^-r_{24}^-$ as a permissive host for T2 amber mutants (Georgopoulous & Revel, 1971) and strain S/6/5 as restrictive host for both T2 and T4 ambers. Strain Bb was used as nonpermissive host for all lysates (Wilson et al., 1970). Strains B/2 and S/4 were used as selective indicators to determine T2 or T4 host range (h^2 or h^4). Identifica-

Table 1

T2:T4 Hybrid Phage

Hybrid Phage	Composition	Host Range
hy1	am123$^+$ amB280$^+$ rdf41	h^2
hy2	am125$^+$ amE2060$^+$ rdf41	h^4
hy11	am123$^+$ amN52$^+$	h^2
hy44	am123$^+$ amC290$^+$	h^2
hy54	am125$^+$ amE2060$^+$	h^4
hy215	am125$^+$ amNG220$^+$	h^4
hy217-5	am129$^+$ amE2082$^+$ rdf41	h^2
hy267	am123$^+$ amNG182$^+$	h^2

The designation am123$^+$ amB280$^+$ for hy1 indicates that hy1 is an am$^+$ recombinant from a cross between T2 am123 and T4 amB280. This same convention is used for all of the hybrids. The position of the T2 and T4 mutations in genes 37 and 38 are shown in Figure 1 and Figure 2a. hy1, hy2, and hy217-5 also carry the rII deletion rdf41.

tion of both $\underline{h}^2$ and $\underline{h}^4$ phage on the same plate was done by plating on BIX, a 3:1 mixture of S/6/5 and B/2, each at about 2×10^9 cells/ml. On this indicator $\underline{h}^2$ phage made turbid plaques, $\underline{h}^4$ phage make clear plaques.

(b) Media

H broth used for phage and bacterial growth and EHA top and bottom agar used for plating assays were prepared as described by Steinberg & Edgar (1962). Dilution buffer was prepared as described by King (1968). Minimal growth medium for preparation of radioactively labeled infected cell lysates contained per liter 7 gm Na_2HPO_4, 3 gm KH_2PO_4, 1 gm NaCl, 1 gm NH_4Cl, 0.12 gm $MgSO_4$, and 4 gm glucose.

(c) ^{14}C-labeled lysates

Strain Bb was grown to 5×10^7 cells/ml. in minimal growth medium, collected by centrifugation, and resuspended at $2\text{-}4 \times 10^8$ cells/ml. One ml. aliquots of this suspension were warmed to 37°C, infected with phage at a multiplicity of 4, and aerated by agitation on a rotary shaker. 14 min after infection 2 μC of a uniformly-labeled ^{14}C amino acid mixture (Schwarz-Mann) were added. At 45 min the lysates were added to an equal volume of cold 10% TCA and dialyzed against 0.065 M Tris-HCl, pH 6.8, 1% SDS.

(d) Electron microscopy

Electron microscopic techniques for examining heteroduplex DNA are described in Davis et al. (1971), and Westmoreland, Szybalski & Ris (1969). Details of our procedure are given in Wilson, Kim & Abelson (1972). Briefly, the heteroduplex DNA was prepared by alkaline lysis of a mixture of two phages and renaturation of the DNA in the presence of

formamide, stained with uranyl acetate, and shadowed with platinum-palladium. Under these conditions single-stranded DNA is extended into a measureable form and appears thinner and kinkier than does double-stranded DNA. Both single- and double-stranded ØX174 DNA (5200 bases long, N. Davidson, personal communication) were added as internal standards.

The crossover points (XOP's) of the hybrid phages were determined by making heteroduplexes of their DNA with that of T4B rH23 and T2L rH23 or, if the hybrids carried the rII deletion rdf41, with that of T4B and T2L. The rII deletions served as markers to orient the heteroduplexes.

(e) Gel electrophoresis and autoradiography

Procedures for the preparation and running of discontinuous polyacrylamide gels containing SDS were as described by Laemmli (1970). Sample preparation, staining, destaining, and autoradiography of the gels were as described by Wilson & Kells (1972). For molecular weight determinations the gels were standardized as described by Beckendorf & Wilson (1972).

(f) Phage crosses

Standard phage crosses were a modification of the procedure of Steinberg & Edgar (1962). T4D crosses were done at 30°C in CR63. Crosses involving T2L or its mutants as one or more parents were done at 25°C in CR63$r_6^-r_{24}^-$. A stationary culture of the host cells was diluted 1:1000 or 1:500 in H broth and grown for 2.5 hr at 30°C. Cells were collected by centrifugation, resuspended at 4×10^8 cells/ml., and KCN was added to an equal volume of the parental phage strains at a multiplicity of 7.5

each at 30°C or 25°C. After 10 min anti-T4 serum, anti-T2 serum, or a mixture of both sera was added to inactivate all unadsorbed phage. 15 min after infection the cells were diluted 1-4 x 10^4 into H broth at 30°C or 25°C. 90 or 110 min after infection $CHCl_3$ was added.

(g) Conventions

All maps are represented as they would appear if viewed from the center of the circular T4 genetic map (see Mosig, 1970, for a recent version of this map). Thus gene 37 is to the left of gene 38. To facilitate comparisons of maps constructed by various means, the positions of markers are often given in fractional parts of T4 gene 37, measured from its left end. As will be seen in section 3.(d), synthesis of P37 begins at the left end of gene 37.

3. Results

(a) Genetic maps of genes 37 and 38

The genetic map of T4 genes 37 and 38 presented here (abscissa Fig. 2a) is a composite of two previous maps. The first, a map of gene 37 presented by Bernstein and Fisher (1968), contains three am sites, amA481, amN52, and amB280, and most of the ts sites in gene 37. The data used to construct this map was generously supplied to us by Harris Bernstein. The second map, which we constructed, contained three gene 37 ts sites, tsL37, tsP43, and tsL93, all of the gene 37 am sites, and the am and ts sites in gene 38. To combine the two maps we crossed am mutants which had been located on our map with ts mutants expected to be nearby.

In this way we determined which ts sites lay in each interval between the am sites on the map we had constructed. The order of the ts sites and their relative spacing within the intervals between am's are essentially as determined by Bernstein & Fisher (1968). The resulting map differs in two important respects from that published by Bernstein & Fisher. We find that tsL19, which is their left-hand terminal marker, is actually in gene 36 not gene 37 (Beckendorf, unpublished). We also find, from the results of two and three-factor crosses, that the ts site defined by tsCB81 is to the left of the tsL37 site rather than to the right of amA481 as published (Beckendorf & Lielausis, unpublished).

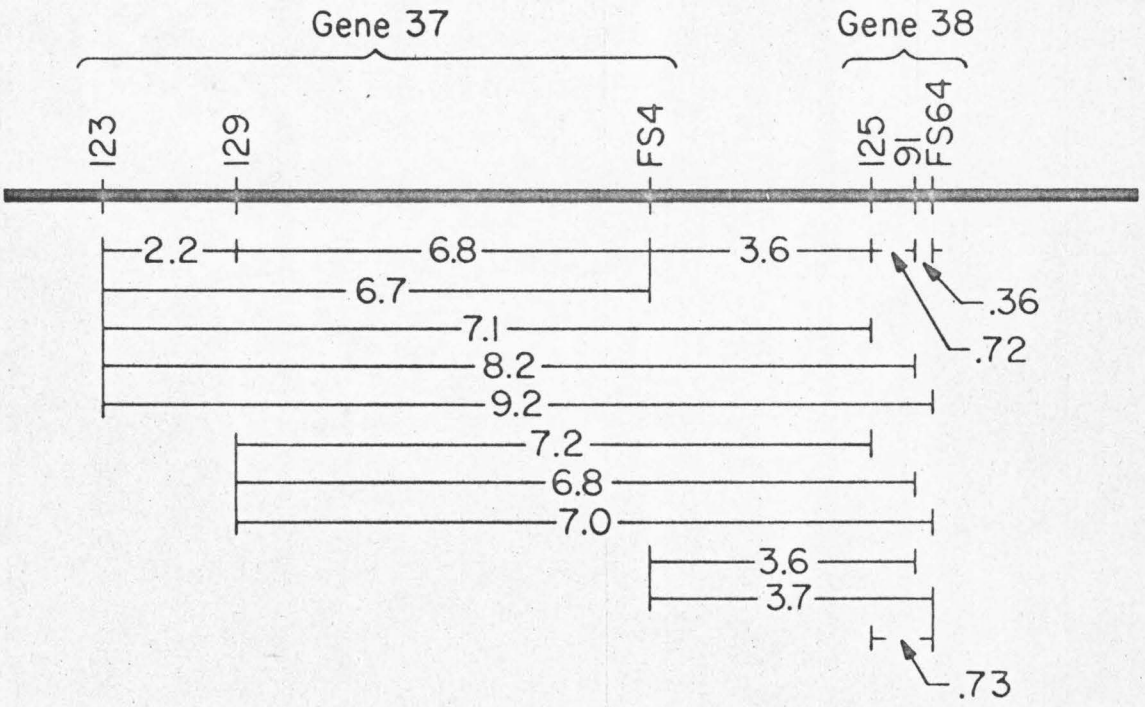
The genetic map of T2 genes 37 and 38 (Fig. 1) was constructed from recombination frequencies determined in two-factor crosses between all of the available am mutants in these genes.

(b) Intertype crosses

We have crossed the ts and am mutants in T4 genes 37 and 38 with the three available am's in T2 gene 37 and am125 in T2 gene 38. The results of these crosses show that most of the recombination occurs in one short segment of gene 37. The crosses have also allowed us to locate the region which controls the difference between T2 and T4 host range.

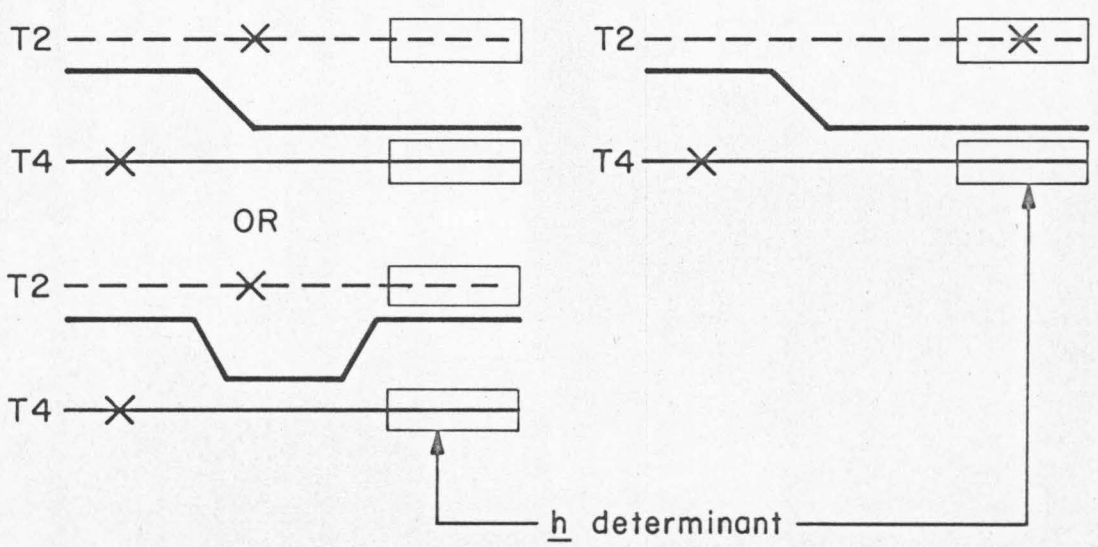
As discussed below, two factors, DNA non-homology and protein incompatibility, might affect the number of recombinants arising in such crosses. Because the DNA molecules would be less likely to pair properly, we would expect a reduction or lack of recombination in nonhomologous regions. The results in section 3.(c) show that most of the T2 and T4 DNA in this region is partially or completely non-homologous.

Fig. 1. Genetic map of T2 genes 37 and 38. The % recombination for each interval is listed below the map and was calculated as ($\underline{am}^+$ recombinants/total progeny) x 200%. All values are the average of recombination frequencies obtained in two independent crosses. All mutants are ambers.


Gene 37
Gene 38
123
129
FS4
125
91
FS64
2.2
6.8
3.6
6.7
.36
7.1
.72
8.2
9.2
7.2
6.8
7.0
3.6
3.7
.73

Protein incompatibilities should also affect the apparent frequency of recombination. The interaction of P37 and P38 is an example of such an incompatibility. Any recombinant in which the interacting sites of the two proteins do not come from the same parental phage will be unable to make tail fibers. As another example, a phage carrying part of gene 37 from T2 and part from T4 might make a protein which would be unable to fold correctly. Either of these examples of protein incompatibility would, like DNA nonhomology, lead to apparent low recombination, since recombinant phage would be unable to form a plaque and would not be scored.

To locate the host range (h) determinant, we have tested whether the $am^+ ts^+$ recombinants have T2 or T4 host range (h^2 or h^4). If, as shown at the left below, the region opposite a mutation is compatible with either host range, both h^2 and h^4 recombinants will occur. However, as shown at the right, if such a region uniquely determines h^4 (is incompatible with h^2), all recombinants will be h^4.



To simplify the discussion of these results, gene 37 has been divided into 4 segments, a, b, c, and d (see Fig. 2a). As will be seen T4 phage carrying mutations in the same segment behave similarly in these crosses.

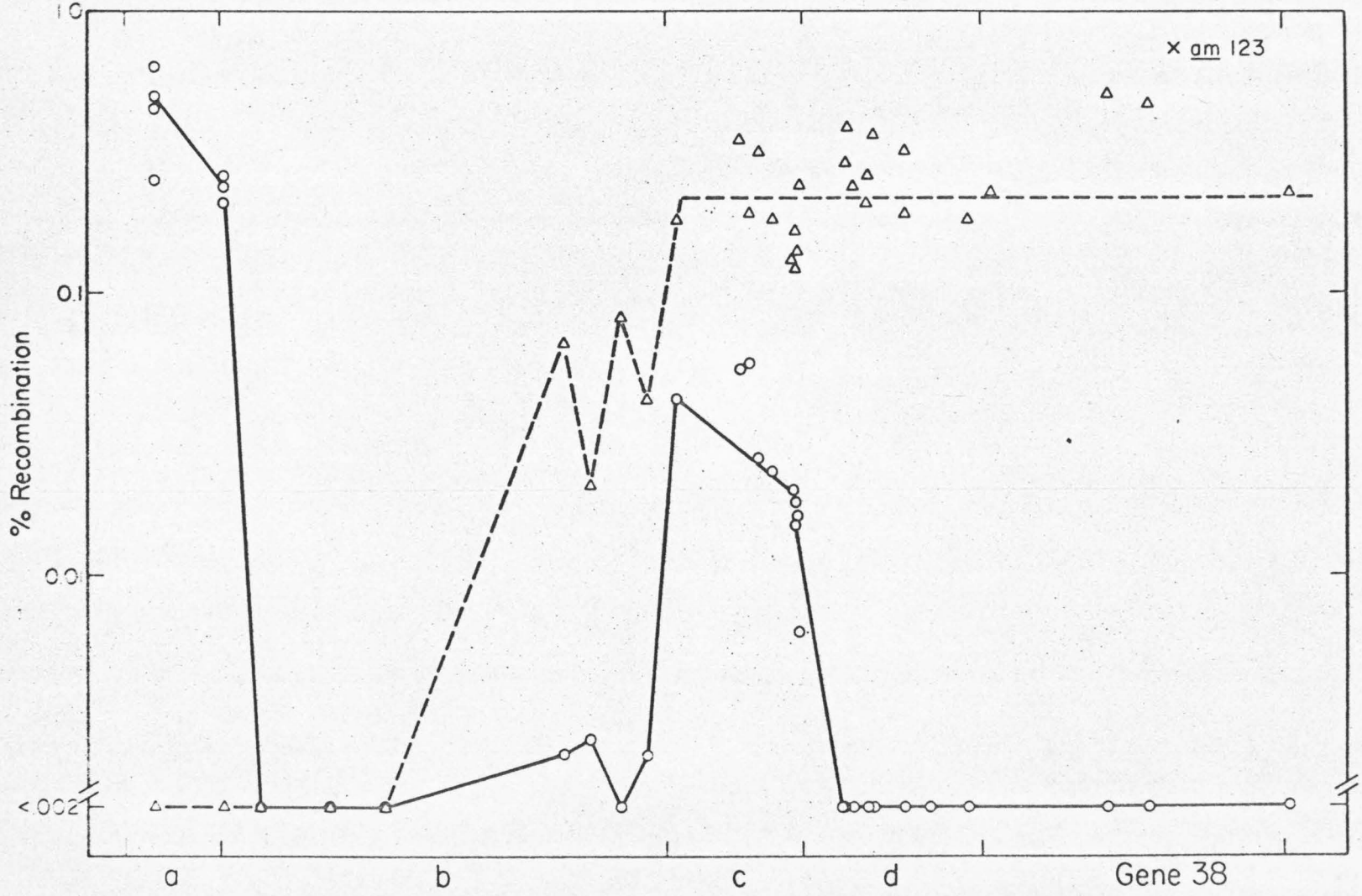
(i) T2 am125 and T2 amFS4 x T4 mutants. Figure 2a and Table 2 show the results of crosses of T2 am125 in gene 38 and T2 amFS4 in segment d of gene 37 with T4 am and ts mutants defective in genes 37 and 38. These crosses gave two types of results. If the T4 mutations were in segment d or gene 38, few if any recombinants were recovered. When the T4 mutants were in segment a, b, or c, $am^+ ts^+$ recombinants were recovered but all were h^4. In none of the crosses were any h^2 recombinants found. Therefore, these crosses are analogous to the second possibility diagrammed above, and the region opposite am126 and amFS4 (segment d and gene 38, Fig. 2a) must determine h^4. Since no recombinants were formed when both the T2 and T4 mutations were in segment d or gene 38, this region in addition to determining host range must either be nonhomologous, specify incompatible polypeptides, or both.

Crosses with T4 mutants in the rest of gene 37 yield about 0.5% of recombinants all of which are h^4. Since these recombinants incorporate at least that part of T2 gene 37 opposite the T4 mutation, the left hand part of the T2 gene, segments a, b, c, must not contain a region which specifies h^2. Since all of the hybrids contain T4 gene 38 and it must interact with a T4 site in gene 37, this site cannot be in segments a, b, or c, but must be in segment d.

In crosses between two T4 mutants or two T2 mutants, the frequency of recombinants increases as the distance between the two mutations

Fig. 2. T2:T4 intertype crosses in genes 37 and 38. Forty *am* and *ts* mutants in T4 genes 37 and 38 were crossed to three *am* mutants in T2 gene 37 and to *am*125 in T2 gene 38 (see legend, Table 2). Each graph represents the results of crossing one or two of the T2 mutants to most of the T4 mutants. Both $\underline{h}^2$ (Δ) and $\underline{h}^4$ (0 or ●) recombinants are represented when present. a. T2 *am*125 (0) and T2 *am*FS4 (●) by T4 mutants. b. T2 *am*123 by T4 mutants. c. T2 *am*129 by T4 mutants. The abscissa of the graphs is the genetic map of T4 genes 37 and 38 constructed as described in the text. The prefix *ts* has been omitted from the designation of all *ts* mutants. % recombination was calculated as ($\underline{am}^+\underline{ts}^+$ recombinants/total progeny) x 200%.

1.0
0.1
0.01
< 0.002
% Recombination
● x am FS4
○ x am 125
L176, N2, N31, CB81
B32, B68, L37
P43
amA481
amNG182
amN52
CB108
amE2060
L20
amE2082
amB280
B78
CI3
L168
N36
B7, B67
B26
NG220
B36
B46
amNG187
B72
amNG475
CB17
CB77, N10
CB89, AI6
A31
L93
CT32
amB262
amC290
CT30
a
b
c
d
Gene 37
Gene 38


1.0
0.1
0.0
< 0.02
% Recombination
× am 123
a
b
c
d
Gene 38

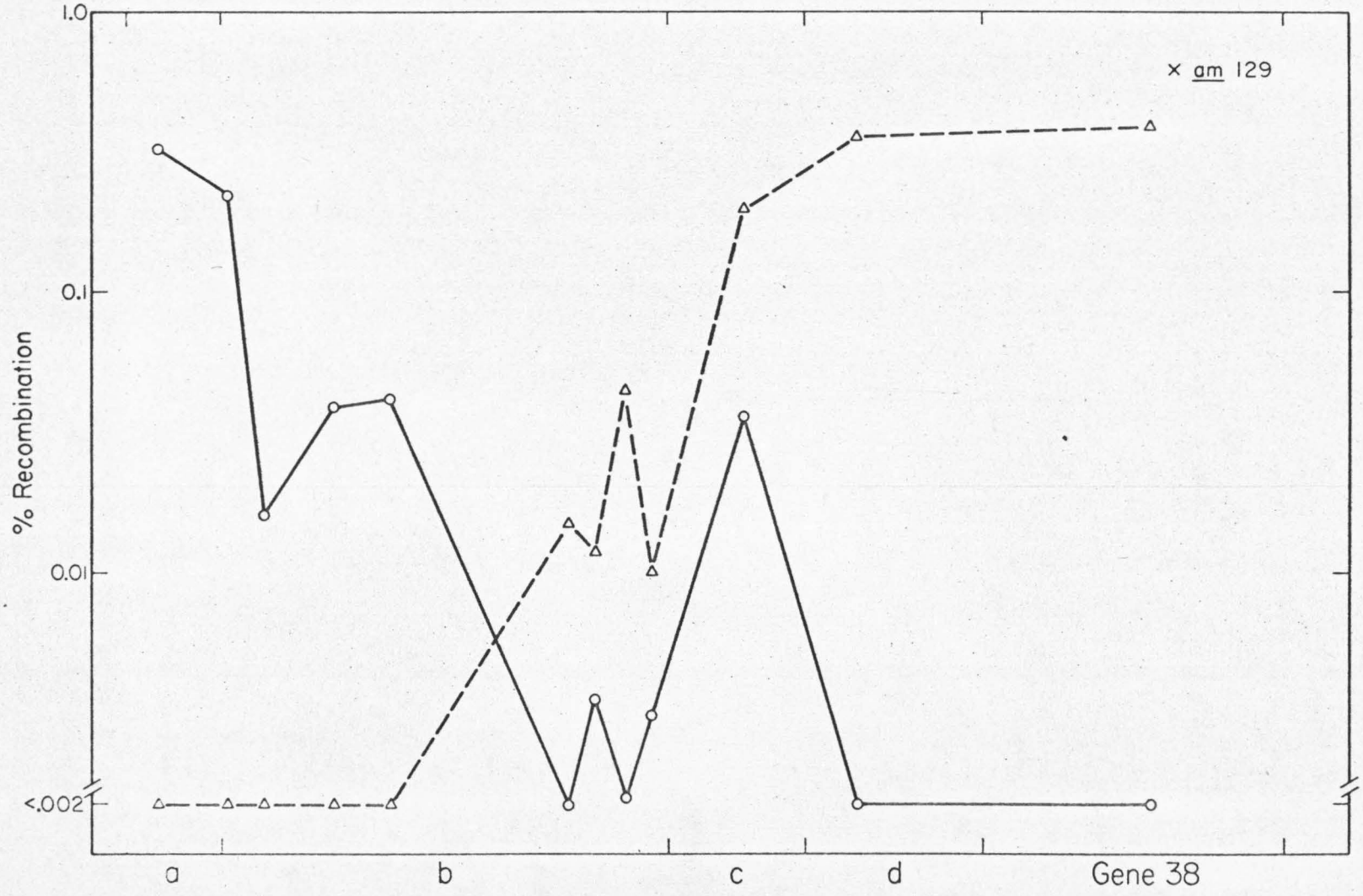

1.0
0.1
0.01
<.002
% Recombination
× am 129
a
b
c
d
Gene 38

Table 2

Intertype Recombination in Genes 37 and 38

T4 mutants	T2 Mutants					
	am125	amFS4	am123		am129	
	h^4	h^4	h^4	h^2	h^4	h^2
tsL176			.45	*		
tsN31			.49	*		
tsN2			.25	*		
tsCB81	.41	.88	.63	*	.32	<.01
tsB32			.26	*		
tsB68	.41		.24	*		
tsL37	.38	.64	.21	*	.22	<.02
tsP43	.18	.53	†	*	.016	†
amA481	.34	.70	†	†	.039	.0017
amNG182	.23	.61	†	.0013	.041	.0019
amN52	.35	.57	.0023	.067	†	.015
tsCB108	.36	.51	.0026	.021	.0035	.012
amE2060	.22	.59	†	.083	.0016	.045
tsL20	.15	.46	.0023	.042	.0031	.010
amE2082	.36		.042	.18		
amB280	.17	.33	.054	.35	.036	.20
tsB78			.057	.19		
tsC13			.026	.31		
tsL168			.023	.18		
tsN36			.020	.13		
tsB7			.015	.12		
tsB67			.018	.16		
tsB26			.016	.14		
amNG220	.040		.0062	.24		
tsB36			†	.29		
tsB46			*	.39		

Table 2 (continued)

T4 mutants	T2 Mutants					
	am125 h⁴	amFS4 h⁴	am123 h⁴	am123 h²	am129 h⁴	am129 h²
amNG187	†	†	†	.24	†	.36
tsB72	†		*	.21		
amNG475	†		†	.26		
tsCB17	†		†	.36		
tsCB77	†		†	.32		
tsN10	†		†	.19		
tsA16	†		*	.18		
tsCB89	†		†	.18		
tsA31	†		*	.23		
tsL93	†					
tsCT32	†					
amB262	*	†	*	.47		
amC290	†	†	*	.51	†	.39
tsCT30	†		*	.23		

Phage crosses were as described in Materials and Methods. A mixture of anti-T2 and anti-T4 serum was used to inactivate unadsorbed phage. All values given are per cent recombination calculated as (am^+ ts^+ recombinants/ total progeny) x 200%. The host range of the am^+ progeny was usually determined by plating the phage on BIX. Occasionally to confirm the host range determined from the BIX plating, plaques were transferred with a sterile pin onto three plates previously seeded with B/2, S/4, and S/6/5 respectively. * <.003% R; † <.001% R.

increases. However in these intertype crosses, the frequency of recombinants remains approximately constant throughout segments a and b. This observation suggests that almost all of the recombination is occurring in segment c and that most of the recombinants carry all of segments a and b from T2.

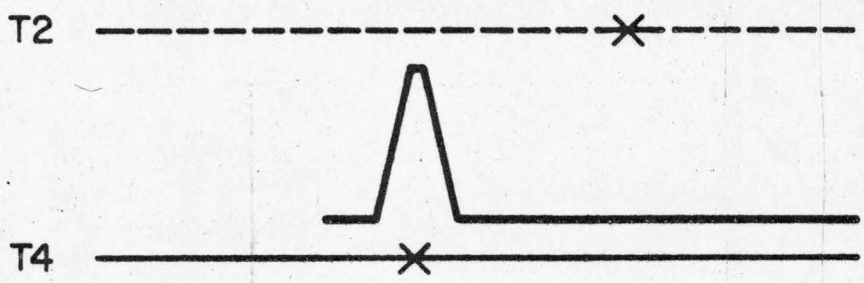
(ii) T2 am123 and T2 am129 x T4 mutants. The results of crosses with these two mutants are very similar (Table 2 and Figs.2b and 2c). In the crosses with am123 the lack of recombinants at the left end of segment b identifies the area on the T4 map opposite am123. No such landmark locates am129, but its position relative to the T4 map can be determined from the molecular weight of the am fragment produced in su$^-$ cells infected with am129 (see section 3f). This fragment of 37,000 daltons locates am129 opposite the gap on the T4 map between amNG182 and amN52 (see Fig. 5).

The finding that crosses of either am123 or am129 by T4 mutants defective in gene 38 or segment d of gene 37 yield only h^2 recombinants, confirms that this region controls host range.

In crosses of am123 and am129 with T4 mutants defective in segment c both h^2 and h^4 recombinants occur. Thus the polypeptides coded by this region must be functionally interchangeable.

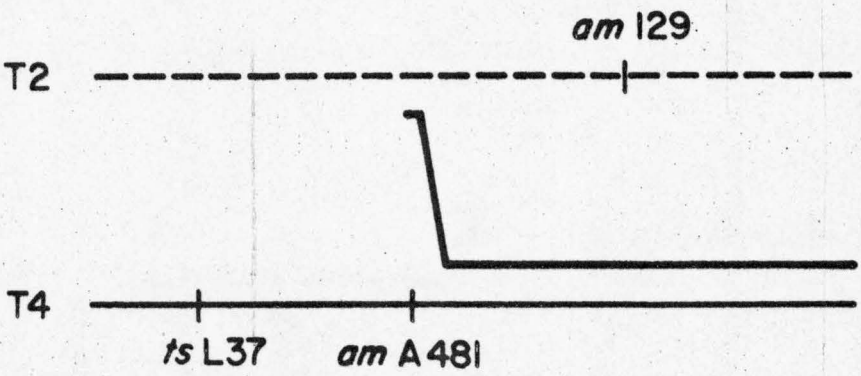
When recombination is forced to occur in segment b because both the T2 and T4 mutations are located in b, the number of recombinants found ranges from 5-30% of the number found when recombination can occur in other regions as well. One possible explanation of this low recombination is that the polypeptides made by this region are partially incompatible. This partial protein incompatibility would allow a small piece of

T2 protein to be incorporated from this region, but any large T2 piece combined with the predominantly T4 molecule would, according to this model, render the protein inactive. Thus to produce an active molecule two crossovers would have to occur, one on each side of the T4 marker.



This requirement for a double crossover would account for the decreased recombination in b.

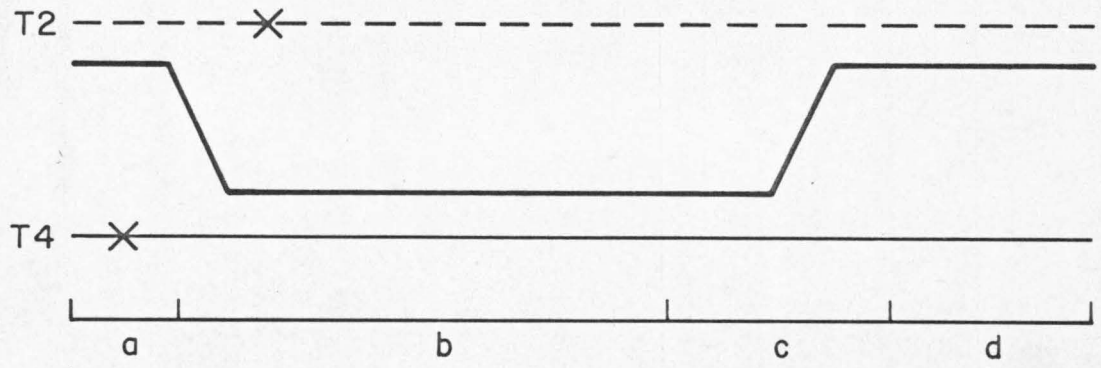
To test this hypothesis, the T4 double mutant tsL37:amA481 was constructed and crossed to T2 am129.



am$^+$ recombinants were selected and tested for the presence of the ts marker. If protein incompatibility were causing the low recombination, a large majority of the am$^+$ recombinants should carry tsL37. The results showed that only 4.1% of the am$^+$ recombinants were also ts. Thus the most likely explanation for the low recombination in segment b is that the DNA molecules are partially nonhomologous. Direct evidence for partial

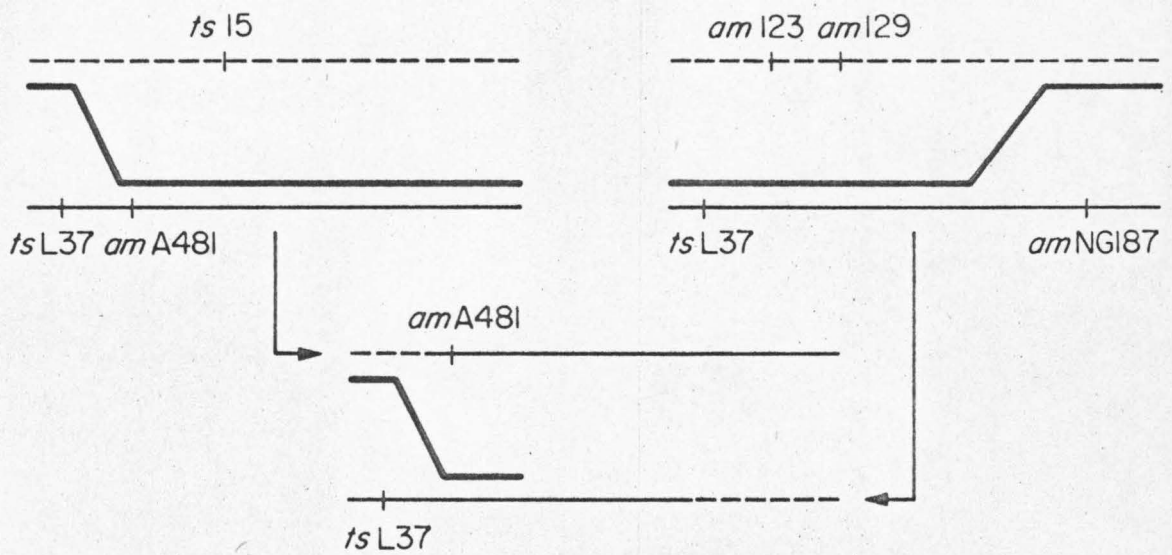
nonhomology in this region is presented in section 3c.

When the T4 mutations are in segment a the total number of recombinants is similar to that in c and d, yet no h^2 recombinants have been found. Since both am123 and am129 are in segment b, recombinants in these crosses must incorporate T2 segment a and T4 segment b. The lack of h^2 recombinants would be explained if the combination of the T2 polypeptide coded by segment a with the T4 polypeptide coded by segment b caused the phage to have T4 host range. Alternatively, since the formation of h^2's in these crosses requires a double crossover, the double



recombinants might be so infrequent that they were not detected. To distinguish these possibilities we constructed two hybrid strains. am^+ts^+ recombinants from a cross between these strains will contain T2 segment a and T4 segment b, but h^2 recombinants will be formed by a single crossover, h^4 recombinants by a double (see Fig. 3). When these strains were crossed 29% of the progeny were $am^+ts^+h^2$ and 15% were $am^+ts^+h^4$. These results

Fig. 3. Construction of hybrid phage for test of recombination between segments a and b. The dotted lines represent T2 gene 37 and the thin solid lines represent T4 gene 37. The heavy solid lines represent the selected recombinant. T2 ts15 maps near am129 and to its right. In the cross T2 ts15 x T4 tsL37:amA481, ts^+ recombinants were selected and tested for the presence of amA481. In the cross T2 am123:am129 x T4 tsL37:amNG187, am^+ recombinants were selected and tested for the presence of tsL37. In the final cross am^+ts^+ recombinants were selected and scored for host range by plating on BIX.


ts 15
am 123 am 129
ts L37 am A481
ts L37
am NG187
am A481
ts L37

demonstrate that the combination of T2 segment a and T4 segment b does not specify h^4 and suggest that the absence of h^2's in the previous crosses was due to nonhomology in segment b which prevented double recombinant formation. In the test cross just described double recombinants ($am^+ ts^+ h^4$) were formed at a high frequency, probably because segments b and c of the parental phage were homologous.

(c) DNA heteroduplex mapping of the host range region

Heteroduplex DNA from a mixture of T2 and T4 phage (T2/T4 heteroduplex) shows a characteristic pattern of substitution and deletion loops when examined in the electron microscope. This pattern of loops has been oriented relative to the standard T4 genetic map by using deletions of genes e and rII as markers (Kim and Davidson, 1972). Plate 1 is an electron micrograph of the region near genes 37 and 38. The top line of Figure 4 is a schematic representation of the loop pattern in this region. Loop 4 is present in all T2/T4 heteroduplexes. In contrast loops 2 and 3 are present in only about 50% of the heteroduplexes and are quite variable in size. Occasionally loops 1 and 2 or loops 2 and 3 open up together. Unless it is combined with loop 2, loop 1 is present in all T2/T4 heteroduplexes. These results indicate that from the right end of loop 1 to the right end of loop 3 the T2 and T4 strands are partially nonhomologous. In loops 1 and 4 the T2 and T4 strands appear completely nonhomologous by this technique, while in the regions between 0 and 1 and between 3 and 4 they appear completely homologous.

By making heteroduplexes between T2 or T4 and the phages which have a hybrid gene 37, it is possible to map the crossover points (XOP's) of the hybrids onto the heteroduplex loop pattern, as shown by the following examples. hy54 is an am^+ recombinant from the cross T4 amE2060 x

Plate 1. Electron micrograph of the host range region of a T2/T4 heteroduplex. Heteroduplex molecules were prepared and visualized as described in Materials and Methods. Bar represents 1000 Å.

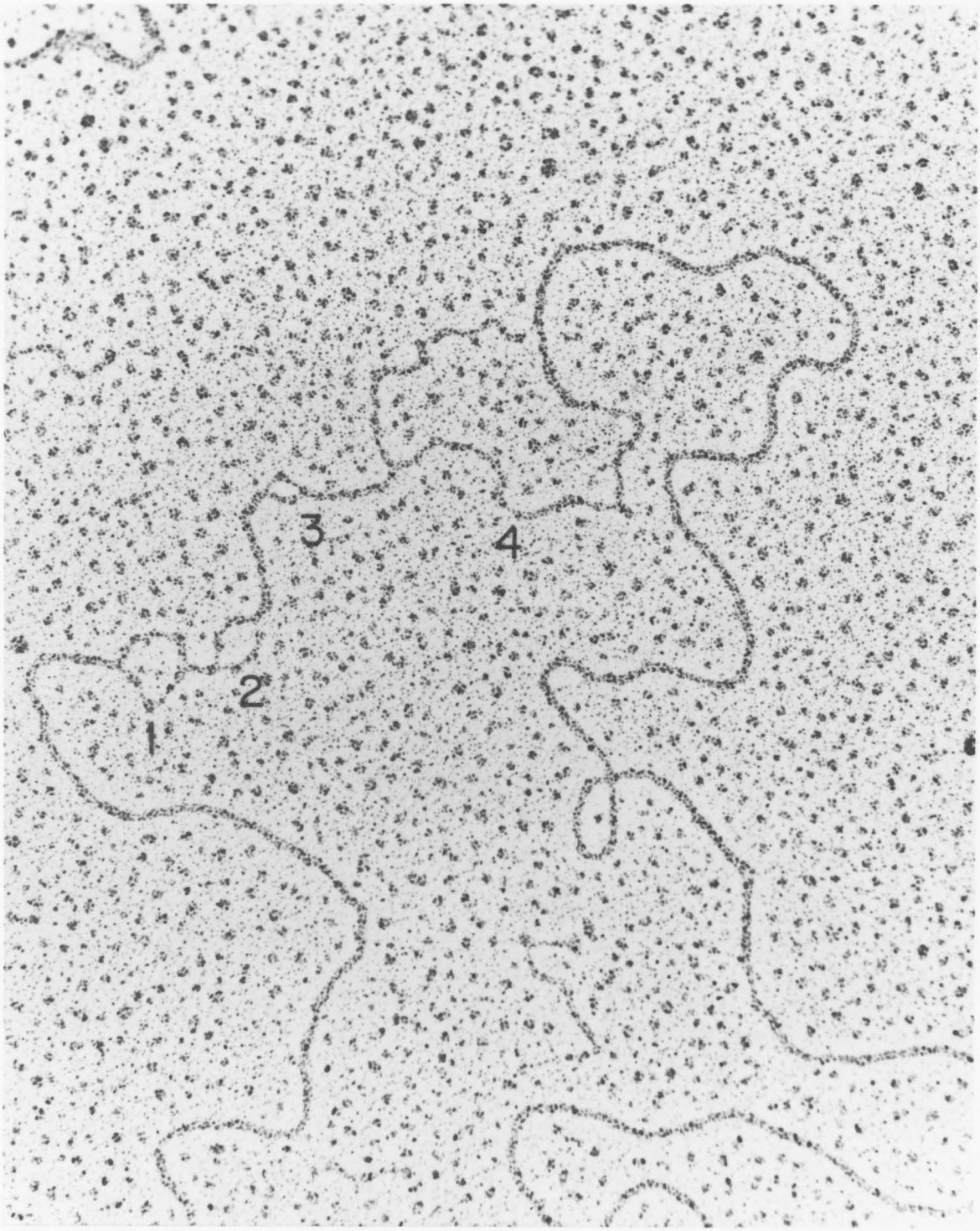
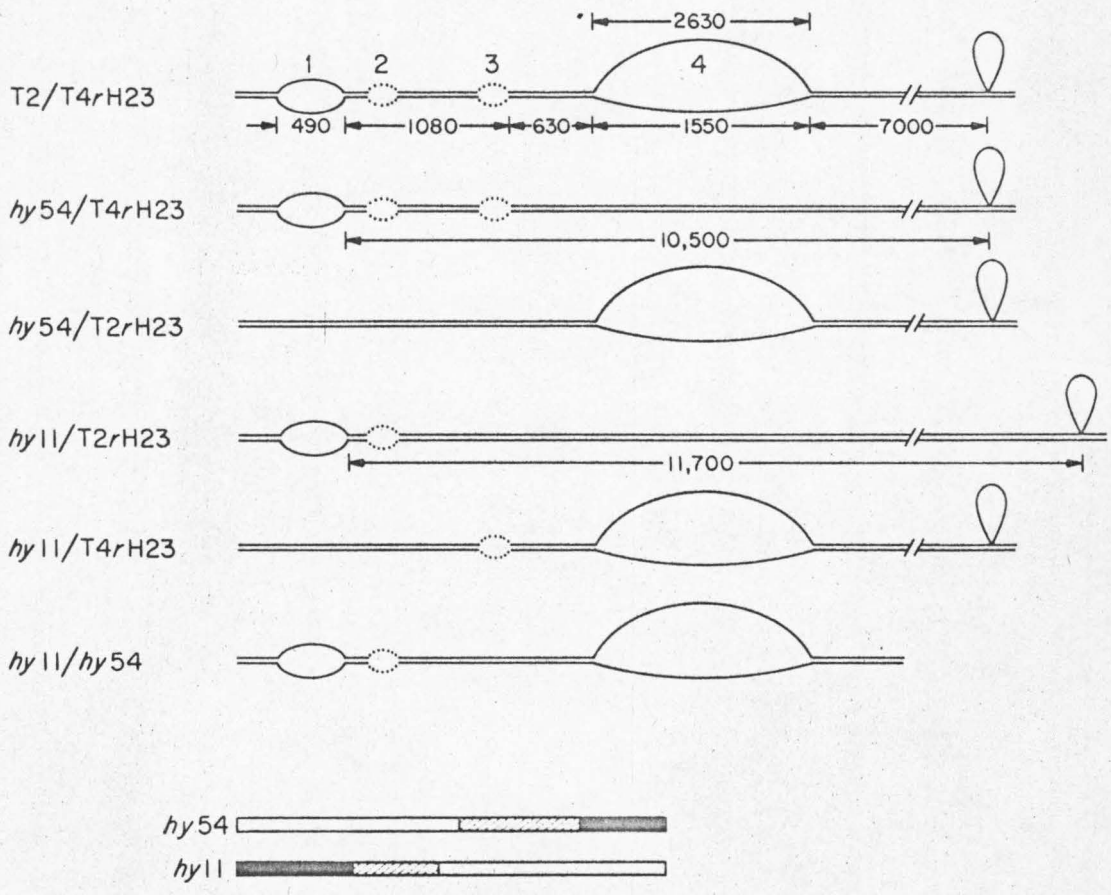

3
4
2
1

Fig. 4. Heteroduplex loop patterns. Heteroduplex molecules were prepared, visualized and measured as described in Materials and Methods. The loops are numbered 1-4 to correspond to Plate 4. Dimensions are given in nucleotides.


2630
1
2
3
4
T2/T4rH23
490
1080
630
1550
7000
hy54/T4rH23
10,500
hy54/T2rH23
hy11/T2rH23
11,700
hy11/T4rH23
hy11/hy54
hy54
hy11

T2 am125. It has T4 host range and from the results in Figure 2a its XOP must be between amE2060 and the h determinant. A heteroduplex between hy54 and T4 rH23 shows loops 1, 2, and 3 but not 4 (Fig. 4). Thus hy54 must be homologous with T4 in the region of loop 4, but not in the region of loops 1, 2, and 3. The heteroduplex hy54/T2 rH23 demonstrates that hy54 is homologous with T2 in the region of loops 1, 2, 3 and confirms that it is homologous with T4 in the loop 4 region. These two heteroduplex patterns locate the XOP in hy54 between loops 3 and 4.

hy11 is an $am^{+}h^{2}$ recombinant from the cross T4 amN52 x T2 am123. Heteroduplexes between it and T2 or T4 indicate that the region of loops 1 and 2 is homologous to T4 and the region of loops 3 and 4 is homologous to T2 (Fig. 4). Then the XOP in hy11 is between loops 2 and 3, confirming our expectation from the genetic results that the XOP's in hy11 and hy54 are at different points. The heteroduplex hy11/hy54 has loops 1, 2, and 4 but is homologous in the region of loop 3 as predicted from the above results.

From the two heteroduplexes hy54/T4 rH23 and hy11/T2 rH23 it is possible to establish that the longer strand in loop 4 comes from T2 and the shorter from T4. In hy54/T4 rH23 the distance from the rII deletion loop to the right end of loop 1 is 10,500 nucleotides. In this case the region corresponding to loop 4 is duplex T4 DNA. In hy11/T2 rH23 the loop 4 region is duplex T2 DNA and the distance from the rII deletion to the right end of loop 1 is 11,700 nucleotides. Therefore the T2 DNA in loop 4 is longer--1200 nucleotides from this calculation, 1080 nucleotides from directly measuring the strands of the loop.

A number of other hybrid phage have been heteroduplexed with T2 or T4 and the resulting loop patterns determined (Table 3). The results

Table 3

Heteroduplexes of Hybrid Phage

Heteroduplex	XOP	Host Range	Number of molecules with				Total number of Molecules
			Loop 1	Loop 2	Loop 3	Loop 4	
hy11/T2rH23	.21-.48	h^2	9	4	0	0	9
hy11/T4rH23			0	0	6	7	7
hy54/T2rH23	.52-.85	h^4	0	0	0	9	9
hy54/T4rH23			23	14	9	0	23
hy11/hy54			9	3	0	9	9
hy1/T4B	.35-.68	h^2	0	0	0	16	16
hy2/T4rH23	.52-.85	h^4	6	4	4	0	6
hy44/T2rH23	.21-.85	h^2	7	4	3	0	7
hy44/T4rH23			0	0	0	16	16
hy215/T2rH23	.80-.85	h^4	0	0	0	20	20
hy217-5/T4rH23	.35-.58	h^2	13	8	5	0	13

The XOP's and host ranges given refer to the hybrid phage in each heteroduplex. The XOP's are expressed as fractions of gene 37 and were calculated from the position of the appropriate am mutations on the genetic and translational maps (Fig. 2a, Table 4). The heteroduplex molecules were prepared and visualized as described in Materials and Methods.

demonstrate that the origin of the DNA in the region corresponding to loop 4 determines the host range of the phage.

The location of XOP's in two different regions of the heteroduplex loop pattern and the correspondence of the h determinant with loop 4 partially align T4 genes 37 and 38 with the heteroduplex map. To directly compare the results of the intertype crosses with the regions of non-homology, we need a physical map of mutations in gene 37 and a precise way of positioning it relative to the heteroduplex loop pattern. These requirements are met in the next section.

(d) Translational mapping of T4 gene 37

Since polypeptide chain termination occurs at the amber codon under nonpermissive conditions, the site of an amber mutation can be physically positioned within a gene by determining the relative sizes of the wild type product and the amber fragment, using the technique of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS). ^{14}C-labeled lysates of T4 and seven different T4 gene 37 am's grown under nonpermissive conditions were analyzed on discontinuous SDS gels (Laemmli, 1970). From autoradiographs of the gels we were able to identify five P37 amber fragments (Plate 2) and, by comparison with a standard curve, to determine their molecular weights (Table 4). Two other fragments, that from amB280 and that from amN52, were not visible apparently because they coelectrophoresed with other bands. To visualize these fragments we constructed multiple mutants carrying either amB280 or amN52 and am mutations in genes whose product was thought to be obscuring the P37 fragment. Gels of these multiple mutants revealed that the amB280 fragment runs at the same position as P20 and the amN52

Plate 2. Identification of P37[4] *am* fragments. ^{14}C-labeled lysates were prepared as described in Materials and Methods and electrophoresed on 7.5% discontinuous polyacrylamide gels containing SDS. After staining and destaining the cylindrical gels were sliced longitudinally. The slices were dried and autoradiographed on Kodak No Screen X-ray film. The differences in absolute migration of corresponding bands in the three groups reflects normal daily fluctuations.

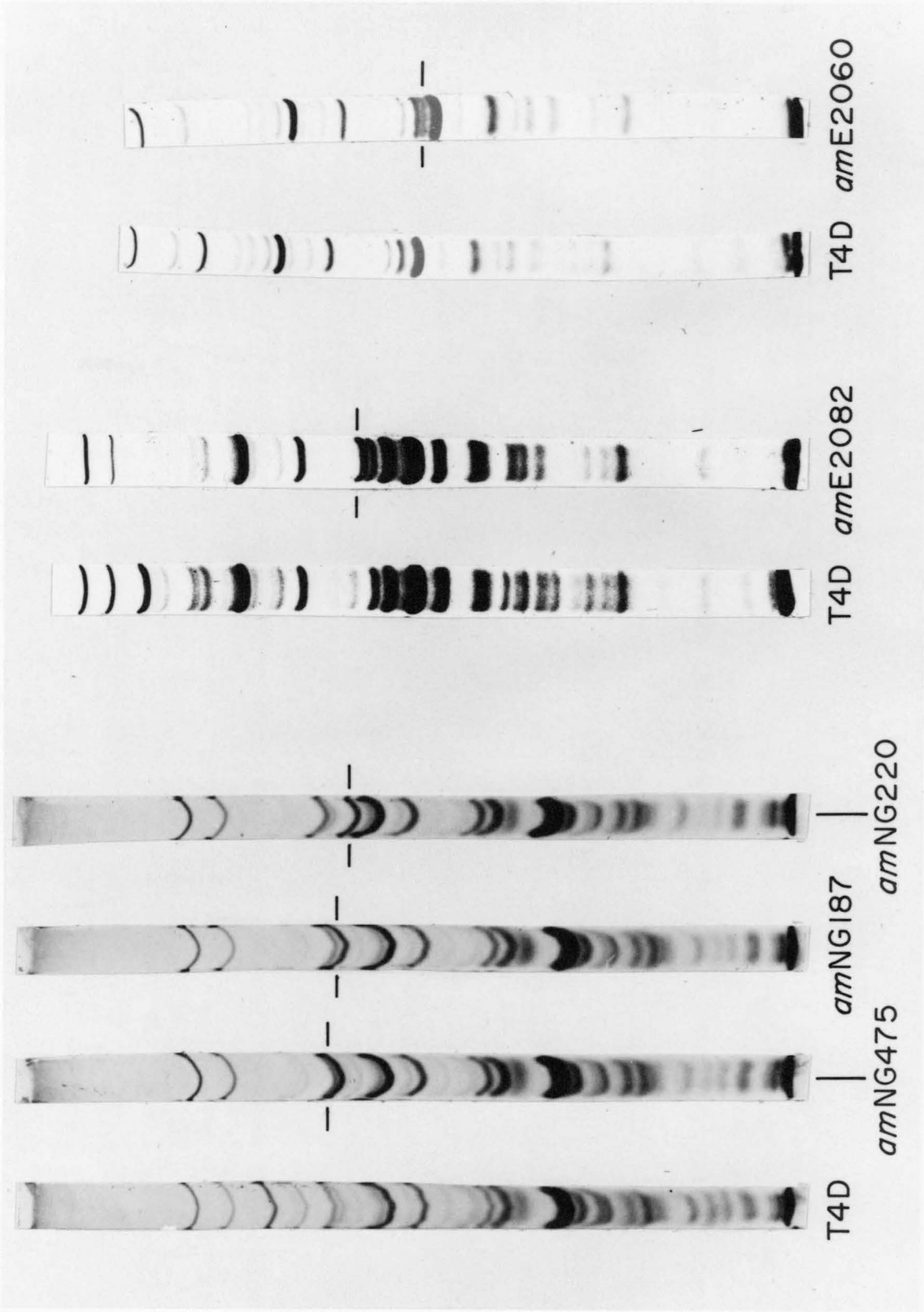

T4D
amNG475
amNG187
amNG220
T4D
amE2082
T4D
amE2060

Plate 3. Identification of amB280 and amN52 fragments in multiple mutants. For procedure see Plate 2. Mutants used: 20^- - amN50; 23^-24^- - amB272 amB26.

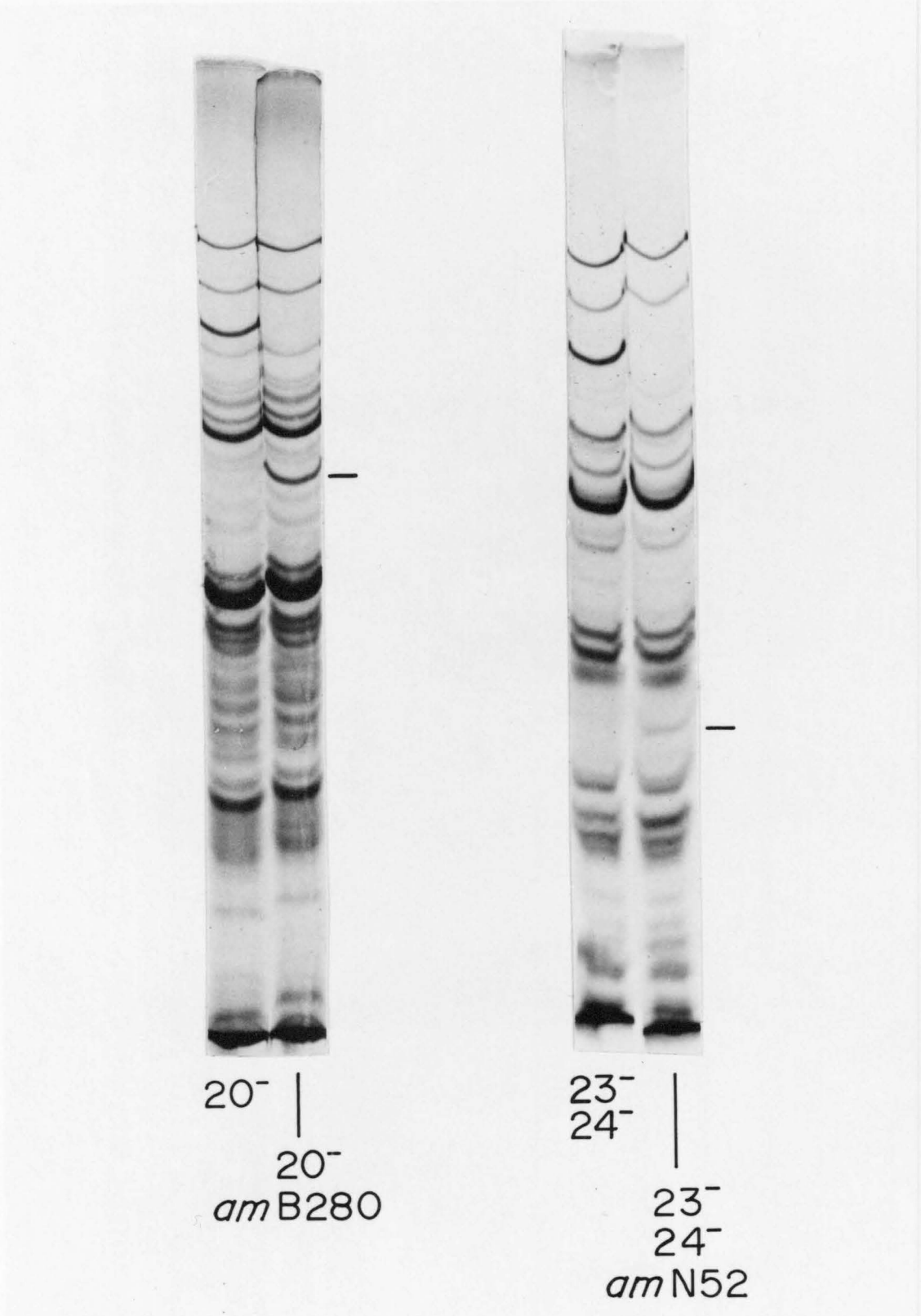

20⁻
20⁻
amB280
23⁻
24⁻
23⁻
24⁻
amN52

Table 4

Molecular Weights of $P37^4$, $P37^2$, and Amber Fragments of Each

	$MW(X10^{-3})$
$P37^4$ *am* Fragments	
*am*N52	50
*am*E2060	55
*am*E2082	61
*am*B280	72
*am*NG220	84
*am*NG187	89
*am*NG475	90
$P37^4$	105
$P37^2$ *am* Fragments	
*am*129	37
*am*FS4	124
$P37^2$	120

Molecular weights of the gene products and *am* fragments was determined by comparing their migration on SDS polyacrylamide gels with the migration of standard proteins as described in Materials and Methods and Beckendorf & Wilson (1972).

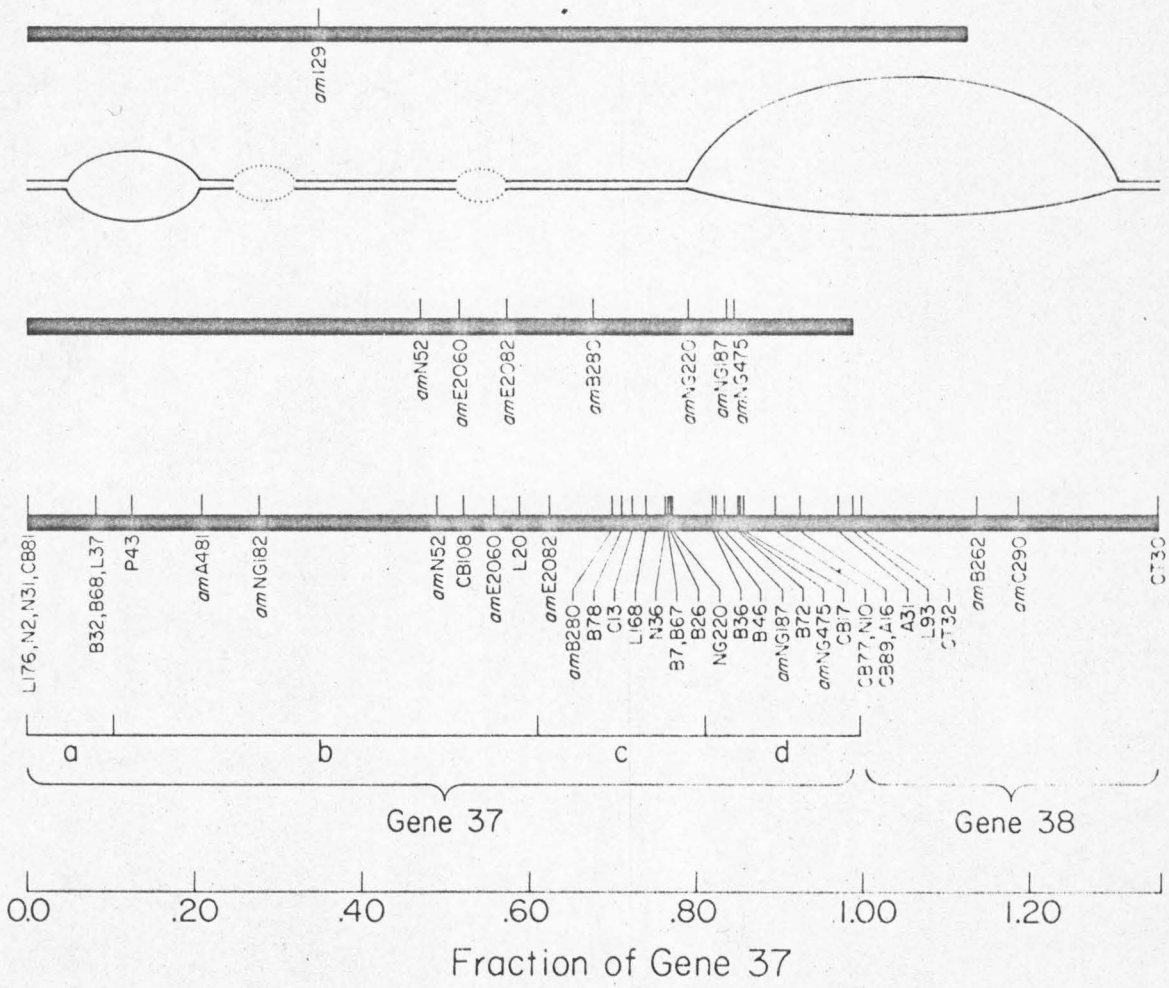
fragment runs very close to the position of P23 (Plate 3). From the molecular weights of these two fragments (Table 4) and the previously determined five fragments, we have constructed the translational map of T4 gene 37 shown in Figure 5.

The nearer the am mutation is to the right end of gene 37, the larger is the size of the am fragment. Thus the direction of translation is from left to right. Studies of the polarity of a gene 36 am mutation (amE1) on the production of P37 and the polarity of a gene 37 am mutation (amN52) on the production of P38 have shown that the direction of transcription of gene 37 is also from left to right (King and Laemmli, 1972).

This translational map can be aligned with the DNA heteroduplex map in the following way. hy215 is an am^+ recombinant from the cross of T4 amNG220 by T2 am125. Therefore amNG220 must be located to the left of the XOP in this hybrid. hy217-5 is an am^+ recombinant from the cross of T2 am129 by T4 amE2082 rdf41; amE2082 must be to the right of the XOP in this hybrid. Heteroduplexes of these hybrids with T2 rH23 show that the XOP's in both hybrids lie between loop 3 and loop 4 (Table 3). Thus both am mutations must also lie between loops 3 and 4. The distance from loop 3 to loop 4 is about 630 nucleotides (Fig. 4). The same figure, 630 nucleotides, is obtained when the interval between the two am mutations is calculated from their separation on the translational map (assuming 3 nucleotides per amino acid and an average residue molecular weight of 110 per amino acid). Therefore we have aligned the translational and heteroduplex maps so that amE2082 coincides with the left end of loop 4.

This alignment permits the left end of gene 37 to be located on

Fig. 5. Comparison of heteroduplex, translational, and genetic maps of genes 37 and 38. From top to bottom the maps are T2 gene 37 translational map, T2/T4 heteroduplex map of genes 37 and 38, T4 gene 37 translational map, T4 genetic map of genes 37 and 38. The scale at the bottom is calibrated in fractional parts of T4 gene 37. The maps were oriented so that the ends of T4 gene 37 were aligned. The prefix ts has been omitted from the designation of all ts mutants.


am129
amN52
amE2060
amE2082
amB280
amNG187
amN5475
L176, N2, N31, CB81
B32, B68, L37
P43
amA481
amNG182
amN52
CB108
amE2060
L20
amE2082
amB280
B78
C13
L168
N36
B7, B67
B26
NG220
B36
B46
amNG187
B72
amN5475
CB7
CB77, N10
CB89, 416
A31
L93
CT32
amB262
amC290
CT30
a
b
c
d
Gene 37
Gene 38
0.0
.20
.40
.60
.80
1.00
1.20
Fraction of Gene 37

the heteroduplex map. The amber fragment of amNG220 is 84,000 daltons, which corresponds to 2310 nucleotides. The left end must then be 2310 nucleotides to the left of the left end of loop 4 or 110 nucleotides to the left of loop 1. By similar calculations the right end of gene 37 is within loop 4, 560 nucleotides from the left end.

The alignment of the translational and heteroduplex maps also determines the positions of the seven translationally mapped am mutants on the heteroduplex map (Fig. 5). To understand the results of the intertype crosses, it would be useful to know the positions of the ts mutants and the other am mutants as well. The genetic map can be used to locate these positions, since the frequency of recombination per unit length is nearly constant throughout the gene. Figure 6 compares the am fragments map with the recombination map by plotting the per cent recombination per 1000 mol. wt. of protein (R_{1000}) for each interval on the translational map. Since the value of R_{1000} varies only slightly throughout the gene (.12-.36), the genetic map can be aligned with the two physical maps as shown in Figure 5.

(e) Translational mapping of T2 gene 37

In SDS polyacrylamide gel patterns of ^{14}C-labeled lysates of T4-infected cells, the three most slowly migrating bands have been identified as P34, P7, and P37 (King and Laemmli, 1972; Ward and Dickson, 1972; King and Laemmli, in preparation). When a gel pattern from T2-infected cells is compared with the T4 pattern, it is clear that bands corresponding to P34 and P7 are present but that no band corresponding to $P37^4$ is present. Instead another band, which migrates more slowly than P7, is present in the T2 pattern (Plate 4). This new band is $P37^2$,

Fig. 6. Comparison of genetic and translational maps of T^4 gene 37. The heavy bar near the bottom of the graph is the translational map of the gene with *am* mutants located along it. R_{1000}, the per cent recombination per 1000 mol. wt. of protein, is plotted for each interval on the translational map.

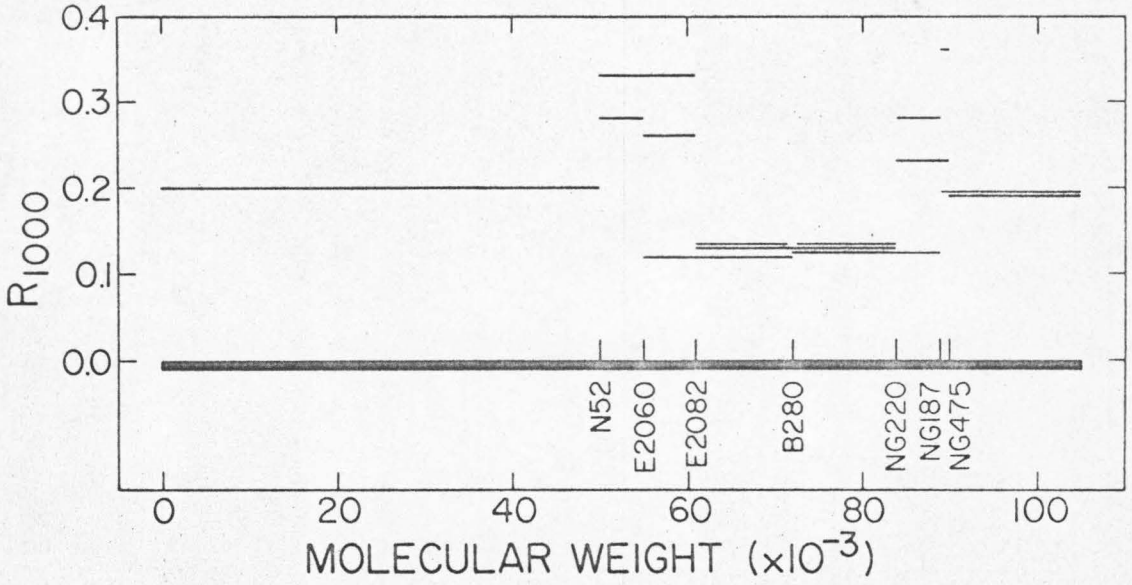
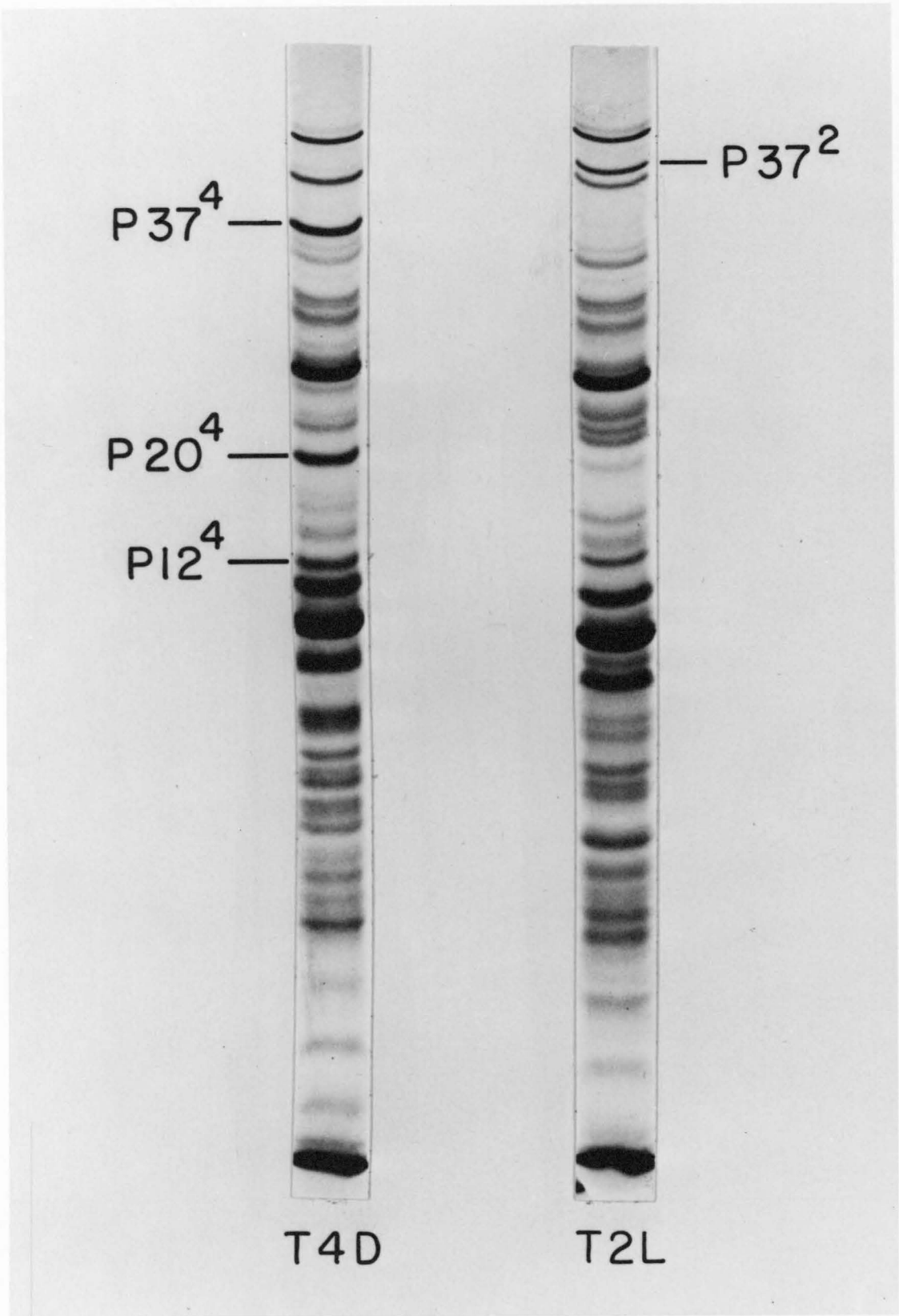

0.4
0.3
0.2
0.1
0.0
R_{1000}
N52
E2060
E2082
B280
NG220
NG187
NG475
0
20
40
60
80
100
MOLECULAR WEIGHT ($\times 10^{-3}$)

Plate 4. Comparison of T4D and T2L proteins. For procedure see Plate 2. Besides the difference in mobility of P37, a number of other bands do not correspond on the two gels, notably P20 and P12.


P37 4
P20 4
P12 4
P37 2
T4D
T2L

as shown by its absence in T2 am123- and am129-infected cells (Plate 5). The difference in the rates of migration of the two polypeptides suggests that $P37^2$ is 13,000 mol. wt. larger than $P37^4$ (Table 4).

The molecular weights of T2 gene 37 am fragments were determined in the same way as the T4 fragments. Two of the three T2 gene 37 mutants produced recognizable am fragments (Plate 5). The molecular weight of the am129 fragment is 37,000 which places am129 opposite the large gap in the T4 genetic map (Fig. 5), as expected from the reversal of h^2 and h^4 frequencies in this region in the crosses of am129 by the T4 mutants (Fig. 2c). The amFS4 gel shows no band at the position of $P37^2$, but there is a band with lower mobility between the positions of $P37^2$ and P34. A gene 22 am fragment with a lower mobility than the wild type product has previously been reported (Laemmli, 1970), but no explanation for this effect has been found.

(f) Location of the molecular weight difference between $P37^2$ and $P37^4$

To determine whether the molecular weight difference between $P37^2$ and $P37^4$ is localized in one part of the gene or dispersed throughout it, we analyzed ^{14}C-labeled lysates made with hybrid phages which have part of their gene 37 from T2 and part from T4. As shown in Plate 6 the P37 from these hybrid phages has either the mobility of $P37^2$ or $P37^4$. Since there are no bands with intermediate rates of migration, the difference in molecular weight must be localized in one region of the gene. As shown in Table 5, the molecular weight difference is controlled by the right end of gene 37. If segment d comes from T2, the P37 band corresponds to $P37^2$; if d comes from T4, the P37 band corresponds to $P37^4$.

Plate 5. Identification of P37[2] and its *am* fragments. For procedure see Plate 2. The banding pattern on the first gel is identical to that of T2L. No *am* fragment can be seen on the *am*123 gel.

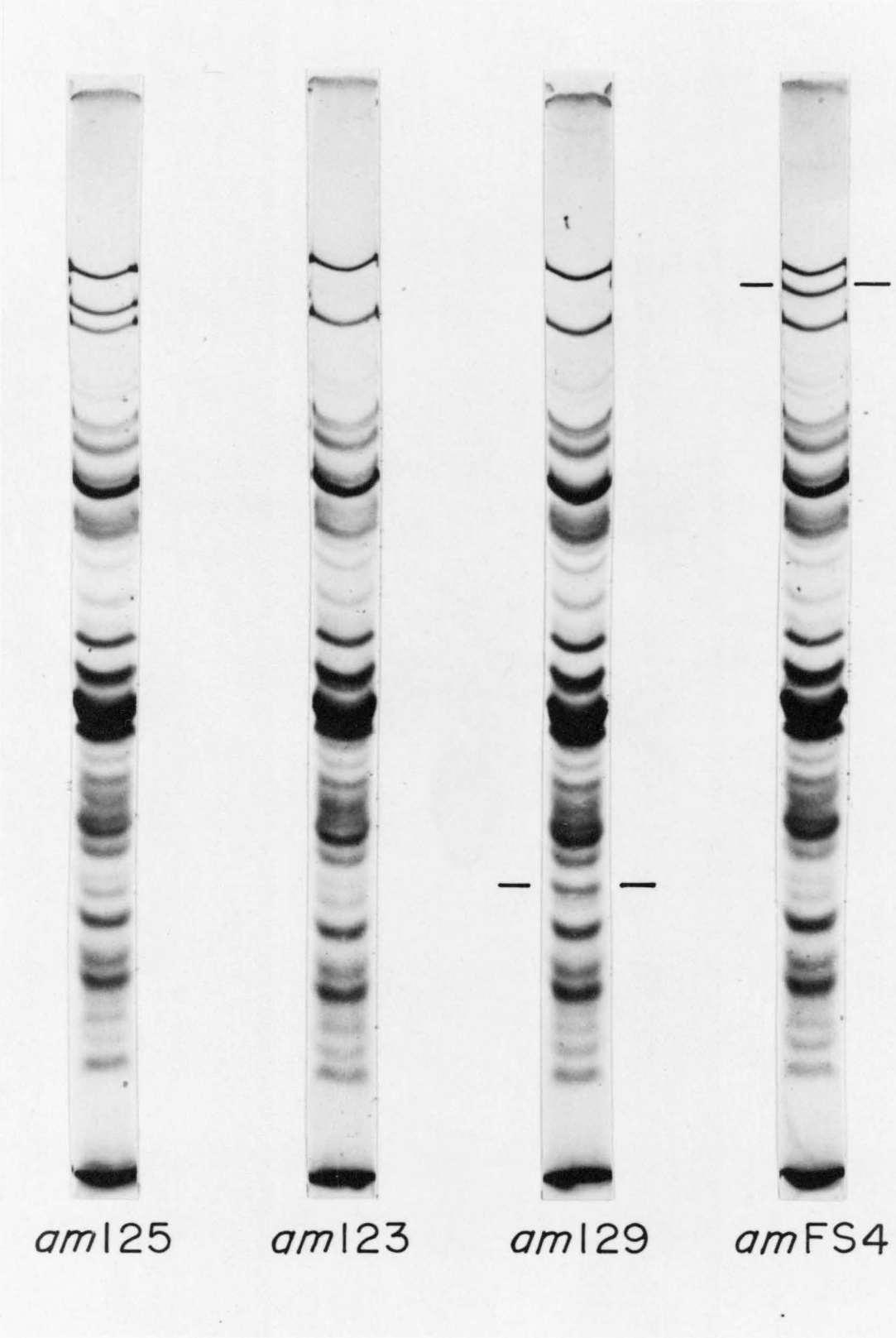

am125
am123
am129
amFS4

Plate 6. Migration of P37 in T2:T4 hybrid phage. For procedure see Plate 2. ^{14}C-labeled lysates of four independent $\underline{am}^+$ recombinants from the cross T2 $\underline{am}$123 x T4 $\underline{am}$C290 were run on the first four gels. ^{14}C-labeled lysates of three independent $\underline{am}^+$ recombinants from the cross T2 $\underline{am}$125 x T4 $\underline{am}$E2060 were run on the last three gels. That the recombinants are in fact hybrids can be seen from the pattern of the lower molecular weight bands (compare with Plate 4). The bars at the bottom represent the composition of gene 37. The filled-in region represents T4 DNA; the open region T2 DNA. The XOP lies within the hatched region.

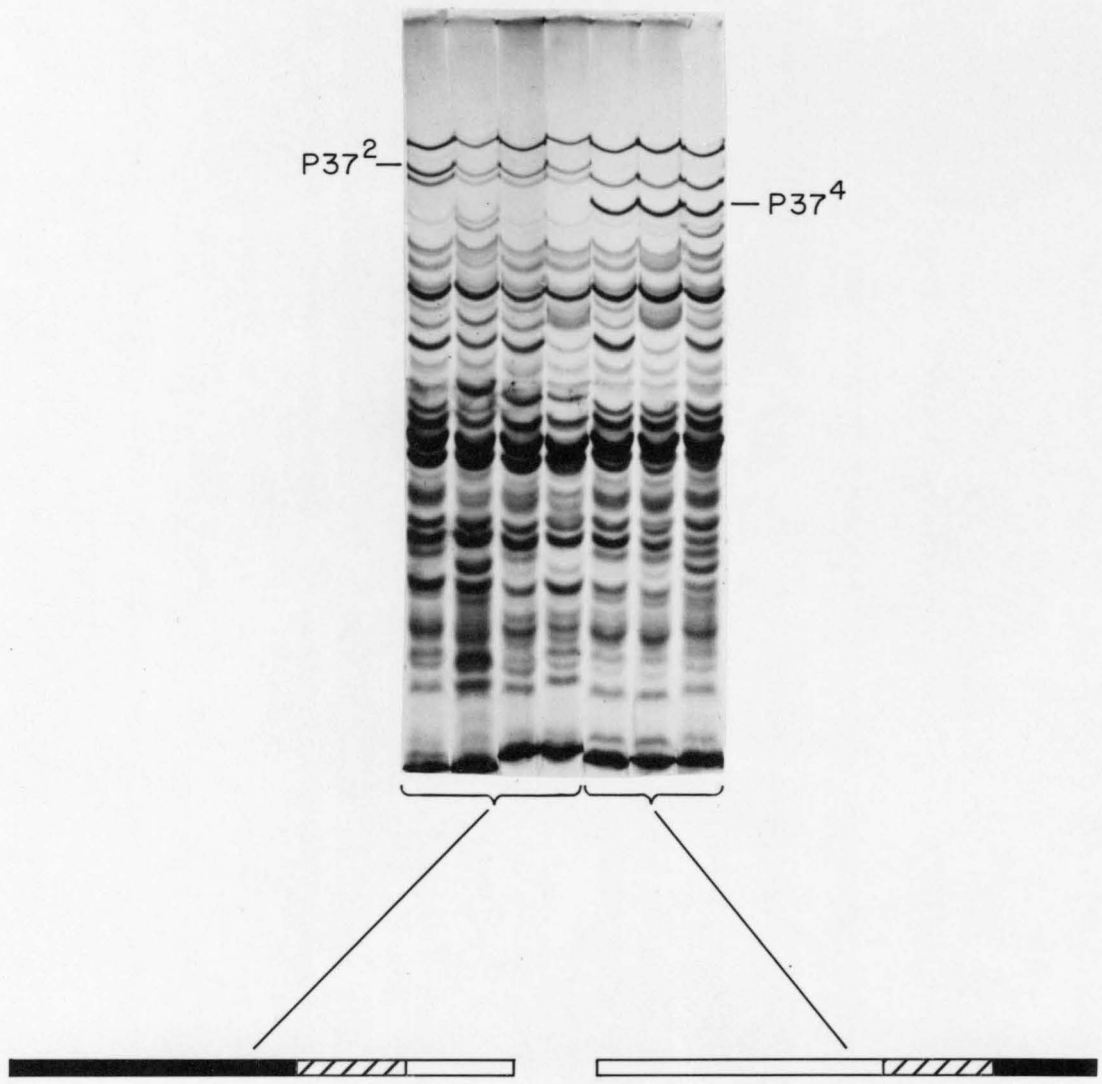

P37^{2}
P37^{4}

Table 5

Migration of P37 from Hybrid Phage

Hybrid	Host Range	XOP	$P37^2$	$P37^4$
*hy*11	h^2	.21-.48	+	
*hy*44	h^2	.21-.85	+	
*hy*54	h^4	.52-.85		+
*hy*215	h^4	.80-.85		+

The XOP's are expressed as fractions of gene 37 and were calculated from the position of the appropriate *am* mutations on the genetic and *am* fragment maps (Fig. 2a, Table 4).

4. Discussion

(a) Recombination frequencies between T2 and T4 markers in genes 37 and 38

It was previously noted that in T2 by T4 crosses recombination between genes 37 and 38 was much lower than expected from the T2 by T2 or T4 by T4 crosses (Russell, 1967). The results in this paper provide some explanations for this low frequency. There are two regions of genes 37 and 38 which in T2 and T4 are nonhomologous. We have not detected recombination in either of them. T2 am123 does not recombine with two T4 mutants which are 1.7 map units apart on the T4 chromosome (tsP43 and amA481, Table 2). Although these mutants have not been physically mapped, they are opposite the nonhomologous loop 1 when the genetic map is aligned with the heteroduplex map (Fig. 5). Similarly, no recombination has been detected between mutants in gene 38 and segment d of gene 37, which correspond to nonhomologous loop 4. Also contributing to decreased recombination in gene 37 is segment b to the right of amA481, in which there is only 5-30% of the number of recombinants as in segments a and c. This low recombination is correlated with partial nonhomology of the DNA in this region.

Thus most of the recombination in genes 37 and 38 in T2:T4 crosses occurs in two relatively short regions, a and c. But even in c the frequency of recombination is strikingly low. It is possible to calculate R_{1000} for two intervals in c using the am fragment molecular weights for amNG220, amB280, and amE2082 and the recombination frequencies from crosses of T2 am125 by these T4 mutants. These calculations give values of 0.011 and 0.017 for R_{1000}, or about tenfold less than for

T4 x T4 crosses in gene 37. This low value might be explained if crosses of T2 x T4 give drastically fewer recombinants in all regions of the map than do the T2 x T2 or T4 x T4 crosses. However, Russell (1967) found that in gene 34 T2 x T4 crosses gave 40 to 50% as many recombinants as did the corresponding T2 x T2 or T4 x T4 crosses. [These results are calculated only for the N-terminal end of gene 34--from amB25 to amN58 in T4 and from am12 to am135 in T2. This part of gene 34 does not show abnormally high values of R_{1000} (Beckendorf & Wilson, 1972).] Since DNA with a small amount of mismatching will appear annealed in the electron microscope, the low recombination in region c might be due to partial nonhomology. Alternatively a normal number of recombinants might be formed but only a few would produce active protein and thus be scored by our techniques. A third alternative is that the nonhomologous and partially nonhomologous regions flanking c might sterically inhibit pairing and genetic exchange in c. This last alternative differs from the first two by not requiring region c to be different in T2 and T4.

(b) Structural and functional observations

Although T2 and T4 are similar enough to be called members of the same species (Russell, 1967), their genes 37 and 38 have diverged sharply. At least 75% of the length of gene 37 and all of gene 38 are partially or completely nonhomologous between the two phages. This nonhomology can be separated into two classes. The functional specificities of gene 38 and segment d of gene 37 have changed during divergence while those of segments a b and c have apparently been unaltered.

Segment d codes for two functions, the interaction of the tail fiber with its specific receptor on the bacterial surface and the interaction

of P37 and P38 during assembly of the tail fiber. Both of these functions are type specific. Whether or not the host range determinant is the same as the site of interaction between P37 and P38 has not been established. Since we have not been able to detect any recombination in d in inter-type crosses, it has not been possible to determine whether the two specificities are genetically separable. Host range mutations of T4 which allow it to infect T4 resistant strains of E. coli K12 map at two very tightly linked sites within segment d rather than throughout the segment (Beckendorf, in preparation).

Along with the functional changes, the physical sizes of segment d and possibly gene 38 have changed during the divergence of T2 and T4. Segment d of T2 is 60% larger than T4 segment d. If we assume that there is no space between genes 37 and 38 and that gene 38 extends from the end of gene 37 to the right end of loop 4, we can calculate a molecular weight for $P38^4$ of 35,000. The molecular weight has been estimated from SDS gels as 26,000 (King & Laemmli, 1972). If T2 gene 38 also occupies the right hand portion of loop 4, its predicted molecular weight would be 60,000.

The functional specificity of segments a, b and c has apparently not changed during the divergence of T2 and T4. In the formation of hybrid phage the T2 and T4 protein coded by these regions has been spliced in a number of places, indicating that the proteins are functionally interchangeable. Since the DNA of segment b is largely non-homologous, it is surprising that splices in b produce active proteins. This suggests that the structure of the protein coded by b is quite simple without much interaction between amino acids which are separated

in the primary sequence.

Despite the sequence divergence of the rest of the gene, segments a and c have been conserved with the same or very similar sequences. It may be that their function is more strictly prescribed than that of segment b. As discussed in the accompanying paper the portion of P37 coded by segment a is located in the assembled tail fiber adjacent to P36 and probably interacts with it (Beckendorf, 1972). Since P36 does not seem to be type specific (Russell, 1967 and Beckendorf, unpublished), the requirement that the portion of P37 coded by segment a interact with P36 might cause its sequence to be conserved. The function of the portion of P37 coded by segment c has not been determined. Two possibilities are discussed at the conclusion of the accompanying paper (Beckendorf, 1972).

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STRUCTURE OF THE DISTAL HALF OF THE BACTERIOPHAGE T4 TAIL FIBER

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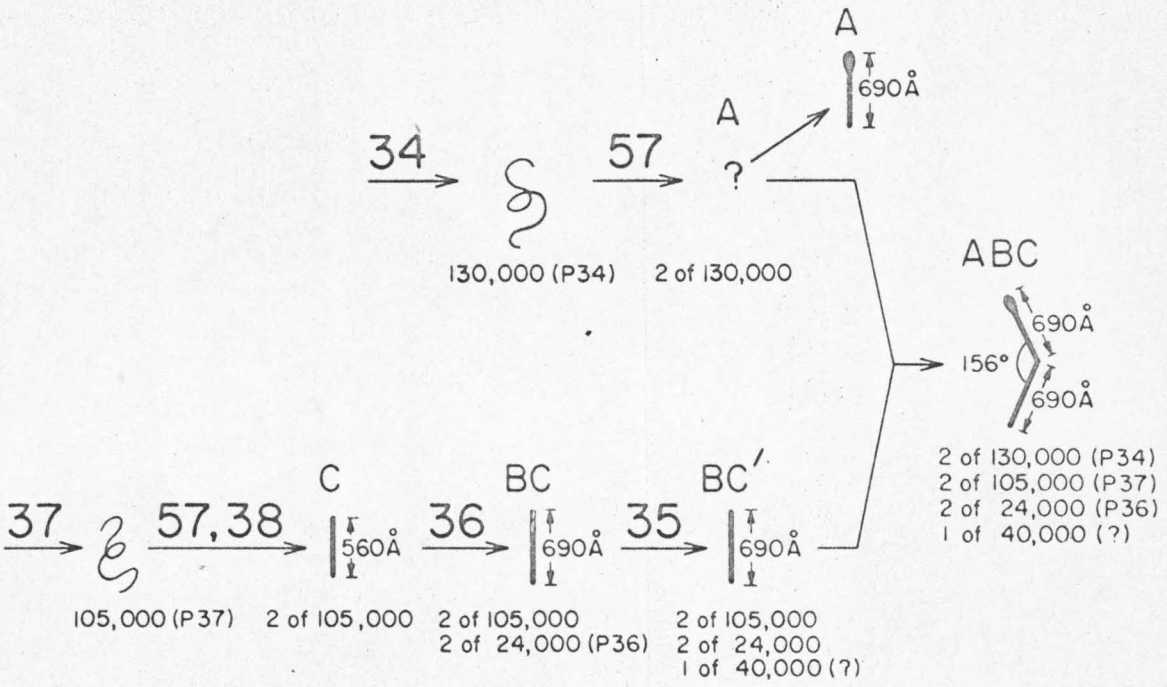
1. Introduction

The assembly of the distal half of the T4 tail fiber requires the action of five genes, as shown in Figure 1. Two antigenic determinants B and C have been distinguished on this half fiber (Edgar & Lielausis, 1965). Genes 37, 38 and 57 act to produce an intermediate which contains two copies of a single polypeptide chain, product of gene 37 (P37) (King & Laemmli, 1971; Ward & Dickson, 197). This intermediate is 560 Å long and carries a serum blocking antigen designated C (Ward et al., 1970). Neither P38 nor P57 are incorporated into tail fibers (King & Laemmli, 1971; Eiserling & Dickson, 1972). The action of gene 36 adds two copies of a second protein, P36, increases the length of the intermediate to 690 Å and adds the B antigen (Ward et al., 1970; King & Laemmli, 1971). The action of gene 35 then adds one copy of a third protein, P35, and prepares the distal half fiber for addition to the proximal half fiber (Eiserling & Dickson, 1972; King & Wood, 1969). The addition of P35 to the BC half fiber intermediate changes neither its length nor its antigens. The final intermediate is designated as the BC' half fiber to distinguish it from the BC half fiber. Finally the BC' half fiber spontaneously combines with the A half fiber to produce the finished tail fiber.

The B antigen is located on the distal half fiber near its joint with the proximal half fiber, while the C antigen is distributed over the rest of the distal half fiber (Yanagida & Ahmad-Zadeh, 1970).

The investigations reported here have had two aims. First we wished to determine whether the C antigen was made up of a number of subsets with different specificities and whether these subsets could

Fig. 1. The pathway of tail fiber assembly. Arrows indicate the steps of assembly under the control of the numbered genes shown above them. The structures shown as intermediates are drawn to approximate their appearance in the electron microscope, their lengths are given, and their antigens are designated above them. The number, molecular weight, and gene product identification of the polypeptides contained in each intermediate are also shown (adapted from Eiserling & Dickson, 1972).


A
690Å
A
34
57
?
130,000 (P34)
2 of 130,000
ABC
690Å
156°
690Å
2 of 130,000 (P34)
2 of 105,000 (P37)
2 of 24,000 (P36)
1 of 40,000 (?)
C
BC
BC'
37
57,38
36
35
560Å
690Å
690Å
105,000 (P37)
2 of 105,000
2 of 105,000
2 of 24,000 (P36)
2 of 105,000
2 of 24,000
1 of 40,000 (?)

be located on the half fiber. To do this we have exploited a series of T2:T4 hybrid phage which have part of their gene 37 from T2 and part from T4 (Beckendorf et al., 1972) and a T4 specific antiserum which will react only with the T4 portion of P37.

Second we wished to determine the orientation of P37 in the assembled half fiber. This has been accomplished by reacting the T4 specific serum with the hybrid phage and observing the resulting tail fiber:antibody complexes in the electron microscope.

2. Materials and Methods

(a) Phage and bacterial strains

Strains of T4D and T2L were as described by Beckendorf et al. (1972). T2:T4 hybrid phage used during this study are described in Table 1.

In addition to the Escherichia coli strains listed in Beckendorf et al. (1972), strain CR63(λ) was used as a selective indicator which does not allow rII mutants to grow.

(b) Media and buffers

H broth used for phage and bacterial growth and EHA top and bottom agar used for plating assays were prepared as described by Steinberg & Edgar (1962). Dilution buffer was prepared as described by King (1968). For electron microscopy sera were diluted in a buffer containing 0.05 M potassium phosphate, pH 7.0, 1.5×10^{-3} M NaN_3. All serum blocking experiments were carried out in SBA buffer (0.01 M sodium phosphate, pH 6.8, 5×10^{-3} M $MgSO_4$, 1.5×10^{-3} M NaN_3, 1 g/l bovine serum albumin).

Table 1

T2:T4 hybrid phage

Hybrid phage	Composition	Host range
hy1	am123$^+$amB280$^+$rdf41	h^2
hy2	am125$^+$amE2060$^+$rdf41	h^4
hy3	am123$^+$am129$^+$tsL37$^+$rdf41	h^4
hy5	am123$^+$am129$^+$amN52$^+$rdf41	h^2
hy11	am123$^+$amN52$^+$	h^2
hy16a	am123$^+$amB280$^+$	h^{2h}
hy44	am123$^+$amC290$^+$	h^2
hy54	am125$^+$amE2060$^+$	h^4
hy172	am123$^+$am129$^+$amE2082$^+$amNG220$^+$	h^4
hy174	am123$^+$am129$^+$amE2082$^+$	h^4
hy176b	am129$^+$amNG182$^+$	h^4
hy215	am125$^+$amNG220$^+$	h^4
hy217-5	am129$^+$amE2082$^+$amNG220$^+$rdf41	h^2
hy217-6	am129$^+$amE2082$^+$amNG220$^+$rdf41	h^4
hy266	am125$^+$amNG220$^+$rdf41	h^4

The designation am123$^+$amB280$^+$ for hy1 indicates that hy1 is an am$^+$ recombinant from a cross between T2am123 and T4amB280. This same convention is used for all of the hybrids. The position in genes 37 and 38 of the T2 and T4 mutations listed here are shown in the accompanying paper (Figs. 1 and 2a). Several of the mutants also carry the rII deletion rdf41. hy16a is a hybrid which is able to infect both B/2 and S/4. For the experiments of this paper it behaves as a normal h^2.

(c) Specific antisera

The anti-T4 BC' antiserum was prepared by injection of rabbits with purified T4 BC' half fibers. Its preparation and properties have been described (Ward et al., 1970). The adsorption of this serum with T2 rH23 and the properties of the resulting serum, AS1, are described in sections of the Results.

(d) Serum blocking assays

A modification of the end point serum blocking assay described by Ward et al. (1970) was used to determine the specificity of the antigens carried by a phage. AS1 was diluted to $k = 0.1\ min^{-1}$ in SBA buffer. Several threefold serial dilutions of the samples to be assayed were made in the serum and incubated 10 to 16 hr at 46 to 48°C. The residual k in each tube was determined by adding a known number of T4D tester phage, incubating for 46 min and then plating the entire contents of the tube with CR63(λ) indicator bacteria to measure the surviving fraction of tester phage. All of the phage whose blocking ability was being assayed carried one of two rII deletions, rdf41 or rH23, and thus were unable to make plaques on CR63(λ). When a sample was expected to block most of the serum, about 800 tester phage were added to each tube. When a sample was expected to block only a small fraction of the serum, about 2500 tester phage were added. The per cent by which the original k is decreased when antigen is in excess is a measure of the per cent of the antibodies which are able to combine with the antigens of the sample.

(e) Electron microscopy

Specimens of the complex between phage and antibodies were

prepared on electron microscope grids essentially as described by Yanagida & Ahmad-Zadeh (1970). One drop of phage suspension (1-5 x 10^{11} phage/ml.) was placed on a carbon-coated Parlodion grid and after about 30 sec washed off with a drop of distilled water. The grid was floated specimen side down on a large drop of antiserum at k = 2.5 and incubated 2-6 hr at 37°C. The specimen was fixed by floating the grid on a drop of 1% glutaraldehyde, 0.5 M potassium phosphate, pH 7.0, for 10-15 min. The grid was then washed with 1 or 2 drops of distilled water and stained with a drop of 2% phosphotungstic acid pH 7.0 for 30 sec. Excess stain was removed with filter paper.

(f) Terminology

The crossover point (XOP) refers to the position at which a genetic exchange took place to produce a recombinant. In this paper the recombinant phage are hybrids between T2 and T4 and the XOP is in gene 37. XOP's are expressed as fractional parts of gene 37 measured from its left end. Because a gene and the polypeptide coded by it are colinear (Sarabhai, Stretton, Brenner & Bolle, 1964), the point on a hybrid P37 at which protein coded by T2 is joined to that coded by T4 has the same fractional value as the corresponding XOP.

In the assembled tail fiber the point at which T2 and T4 protein are joined is defined as the transition point (TP). TP's are expressed as Å between the proximal end of P37 in the distal half fiber (assumed to be 130 Å distal to the joint between the half fibers [see section 3(c)(iii) and Ward et al., 1970]).

P preceding the number of a gene specifies the product of that gene. Superscript 4 or 2 following the number of the gene indicates

that the product came from T4 or T2 respectively. Thus $P37^4$ indicates the product of T4 gene 37.

3. Results

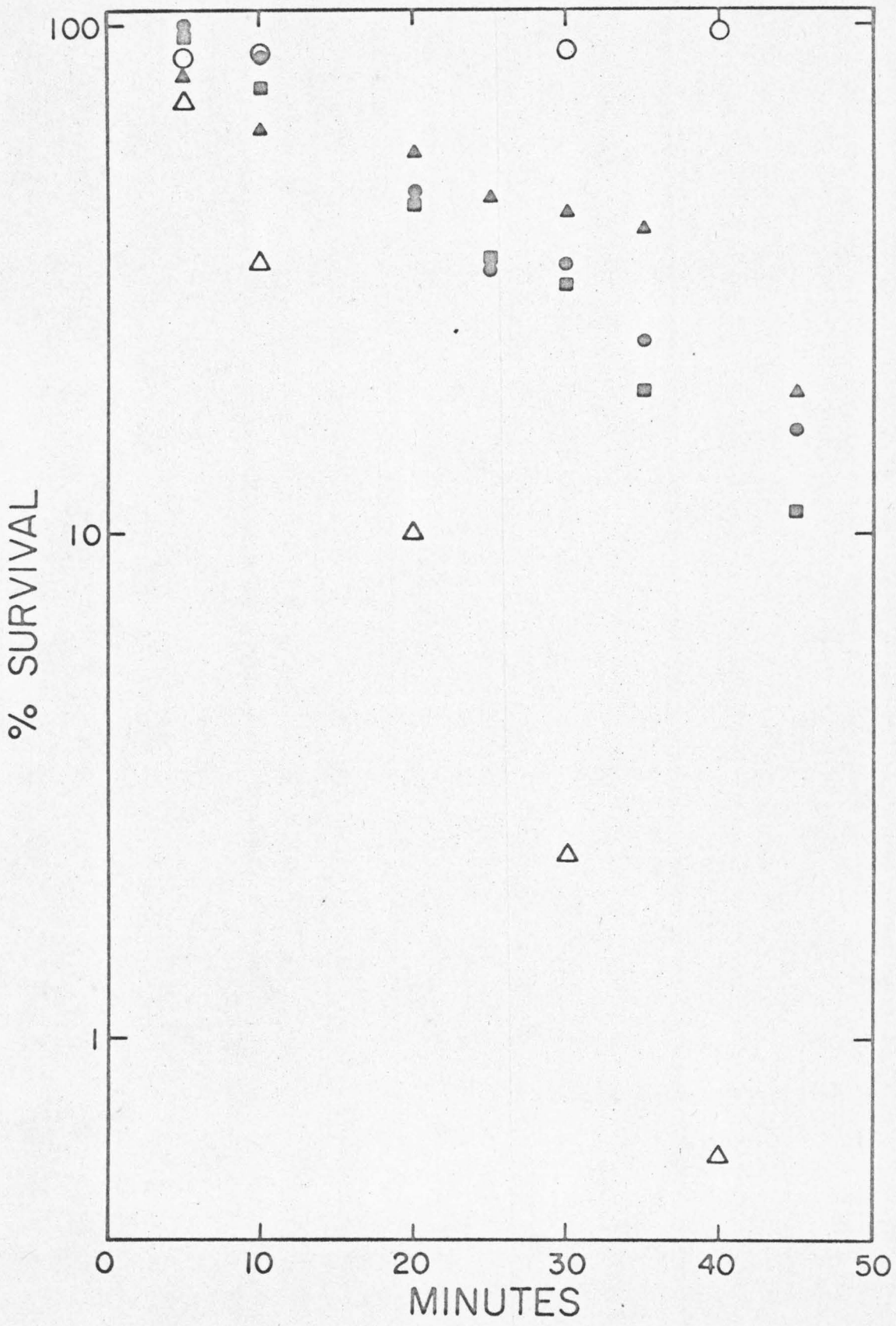
(a) Inactivation of T2 and T4 by anti-T4 BC' serum

To investigate the similarity of the B and C antigens of T2 and T4 we measured the inactivation of the two phages by antiserum prepared by immunizing rabbits with purified T4 BC' half fibers (anti-T4 BC' serum) (Ward et al., 1970). As shown in Figure 2, T4 is rapidly inactivated while T2 is inactivated slowly if at all. Three T2:T4 hybrid phages, which have part of gene 37 from T2 and part from T4 (Beckendorf et al., 1972), were also tested and are inactivated at intermediate rates. Therefore, the distal half fiber antigens of T2 and T4 are not identical and the specificity of the antigens is at least partially determined by the origin of the P37 in the half fiber.

(b) Serum blocking

Two possibilities might explain the decreased rate of serum inactivation of the gene 37 hybrids relative to T4. The C antigen of T4 might be composed of a number of different antigenic sites capable of interacting with different classes of antibodies. These sites would be interspersed throughout the length of P37 so that any segment of $P37^4$ would have the same antigenic specificity as any other. The C antigen of T2 would have a different set of antigenic sites distributed in the same way. A phage with a T2:T4 hybrid P37 would then have a reduced amount of T4 C antigen and would be inactivated at a lower rate than T4. But the hybrid would carry all of the antigenic specificities of the T4 C antigen. This possibility seemed attractive since, besides

Fig. 2. Inactivation of T4, T2, and hybrid phage by anti-T4 BC' serum. At t = 0, 0.1 ml. of a phage suspension was added to 0.9 ml. of SBA buffer containing anti-T4 BC' serum at K ≈ 0.1. The mixture was incubated at 48 ± 2° and at various times aliquots were removed and plated on CR63$r_6^-r_{24}^-$ indicator bacteria. Δ T4D, O T2L, O *hy*11, *hy*44, Δ *hy*54.


100
10
1
% SURVIVAL
0
10
20
30
40
50
MINUTES

P37, two other gene products are necessary for the appearance of C antigen. In the absence of P38 or P57, normal amounts of P37 are made, but do not become antigenic (Ward & Dickson, 1971; King & Laemmli, 1971). It has been suggested that these gene products might modify P37, thereby producing the antigens. If either P38 or P57 modified a number of residues in a similar way, perhaps by adding carbohydrates, the antigenic site on each modified residue would be the same or nearly the same as all others produced by that gene product. Since the C antigen is distributed throughout much of the distal half (Yanagida & Ahmad-Zadeh, 1970), these identical antigenic sites might also be distributed throughout the fiber and presumably throughout the length of P37.

The other explanation for the low rate of inactivation of the gene 37 hybrids is that the C antigen is composed of a number of different antigenic sites and that these are located in discrete units at different locations along P37. Under this explanation any gene 37 hybrid would receive only a fraction of these sites from T4. Thus it would interact with only a few of the classes of antibodies in the serum and would be killed at a lower rate than T4.

These two possibilities can be distinguished by incubating an excess of hybrid phage with the serum until the reaction has gone to completion and then testing the amount of serum activity which has been eliminated or blocked. The first possibility discussed above would predict that the hybrids would block all of the serum activity. The second possibility would predict that the hybrids would block only a

fraction of the serum and that different hybrids would block different fractions.

(i) A T4-specific anti-BC' serum. T2 was inactivated slowly if at all by the anti-T4 BC' serum (Fig. 2). A serum blocking experiment showed that T2 was able to block about 8% of the neutralizing activity of this serum. Since we wanted to investigate the location of the T4-specific antigens on P37 we adsorbed this serum with an excess of T2 rH23 to remove all antibodies able to react with T2. The resulting serum, AS1, is not blocked at all by T2 rH23, but as expected is blocked completely by T4 rdf41.

About 10% of the activity of the unadsorbed serum is directed against the B antigen (Ward et al., 1970; Beckendorf, unpublished). This antigen is added to the C half fiber by the action of P36. Since we were mainly interested in the distribution of the C antigen along P37 and the C half fiber, we tested to see how much of the anti-B activity remained in AS1. A 36⁻ lysate of T4, which contains C but not B antigen, was able to block 89% of the AS1 activity indicating that the adsorption with T2 rH23 had removed about equal fractions of anti-B and anti-C antibodies.

(ii) Serum blocking by gene 37 hybrids. We next tested the ability of several hybrid phages to block AS1 (Fig. 3 and Table 2). Most of the hybrids block only part of the serum activity. Therefore the antigens are not interspersed throughout the length of P37.

The T4 antigens carried by hy5 and hy266 are largely different, since their mixture blocks much more of the serum than either hybrid alone (Table 2). The same can be said for the mixtures of hy5 and hy2

Fig. 3. Blocking of AS1 by hybrids. Serum blocking was carried out as described in Materials and Methods. hy5, ▽ hy266, △ hy217-5, hy5 + hy266, 0 hy217-5 + hy266.

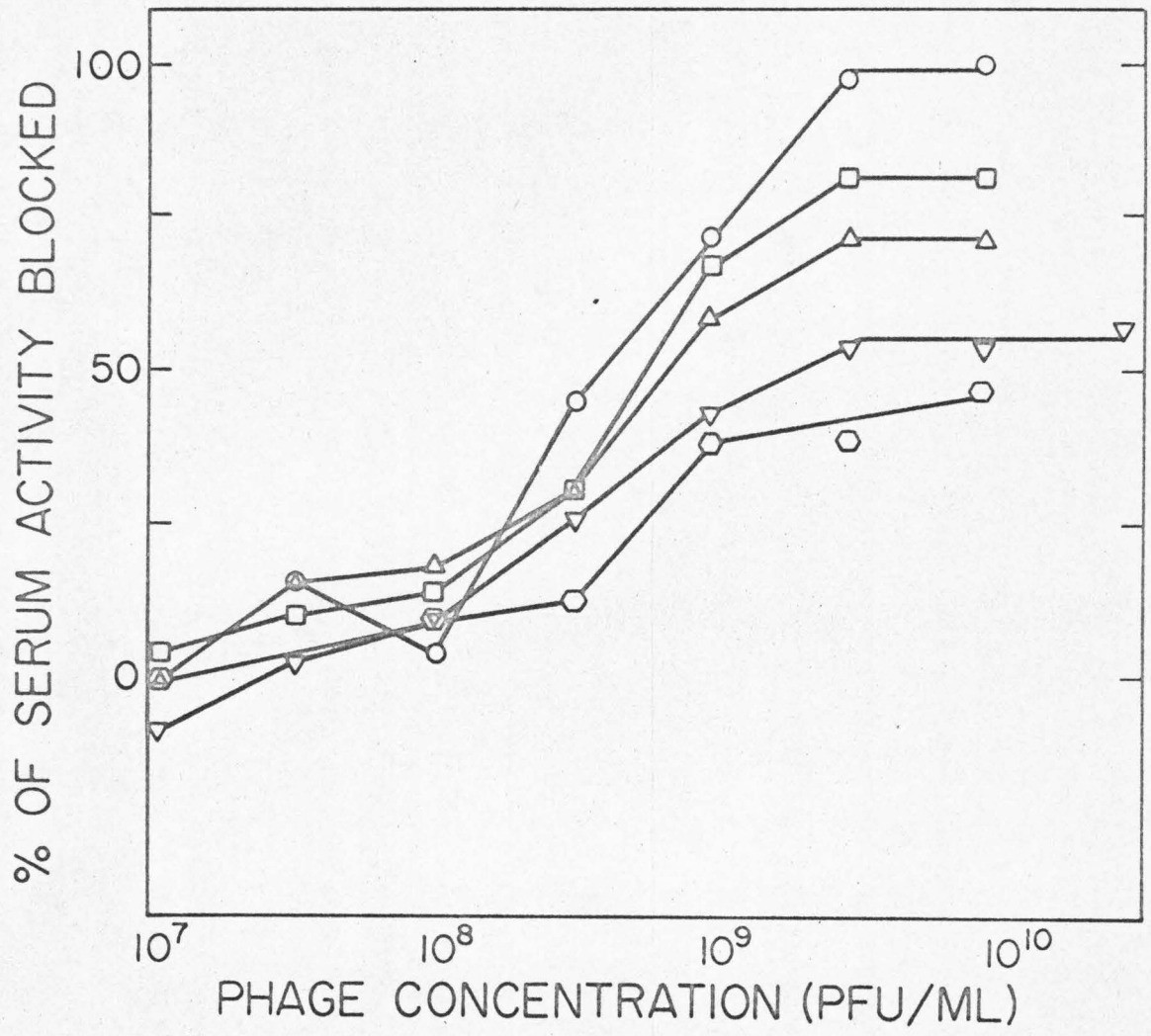
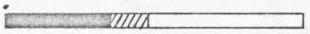
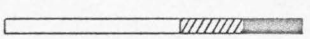
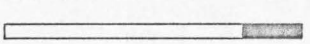
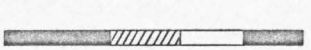

100
50
0
% OF SERUM ACTIVITY BLOCKED
10^7
10^8
10^9
10^{10}
PHAGE CONCENTRATION (PFU/ML)

Table 2

ASl serum blocking by hybrid phage

Phage	XOP		% Blocked
hy3	.08-.13		100
hy5	.35-.48		47
hy217-5	.58		64
hy2	.58-.80		61
hy266	.80		48
hy217-6	.35-58 .80		100
hy5 + hy266			74
hy5 + hy2			91
hy217-5 + hy266			100

Serum blocking was carried out as described in Materials and Methods. The XOP's are expressed as fractions of gene 37 and were determined by genetic and physical mapping (Beckendorf et al., 1972). The bars in column 3 depict the composition of gene 37. The solid region represents T4 DNA; the open region T2 DNA. The XOP is located within the hatched region. The percentage of the serum activity blocked was determined by averaging the results from the two or three tubes with the highest phage concentration (see Fig. 3 and Materials and Methods).

or hy217-5 and hy266. Although there are clearly differences in the antigens carried by the two members of each of these pairs, in no case does the mixture block as much of the serum as the sum of the blocking abilities of the individual hybrids. This result implies that in every case the two hybrids in a mixture carry some antigens in common. Perhaps the two ends of P37 have a common antigen. Another possible explanation is that all of the hybrids carry T4 gene 36 and all are blocking the antibodies specific for the T4 B antigen.

In hy217-6 the region from .58 to .80 fractional length of P37 comes from T2. Yet, despite the fact that 22% of its P37 comes from T2, this hybrid blocks all of the activity of AS1. Therefore, either there are no T4-specific antigens in this region of P37[4] or these antigens are repeated in other regions of P37. A comparison between hy2 and hy266 demonstrates that there are T4-specific antigens in this region. Antigens which react with 13% of the serum are present in hy2 but not hy266. Then these antigens must be duplicated elsewhere in the protein, and since they are not present in hy266, the region in which they are duplicated must be to the left of .58.

If hy5 carried the duplicated antigens the two mixtures hy5 + hy266 and hy5 + hy2 should block the same amount of the serum. However, the first mixture blocks 17% less of the serum than does the second (Table 2). Therefore, hy5 does not contain the duplicated antigens. Since from the previous arguments these antigens must be duplicated to the left of .58, they must be between the XOP of hy5 and .58. The difference in blocking between hy5 and hy217-5 is 17% or approximately the same as the difference between hy2 and hy266 which defined the

duplicated antibodies. Thus the region from the hy5 XOP to .58 may contain only the repeated antigens.

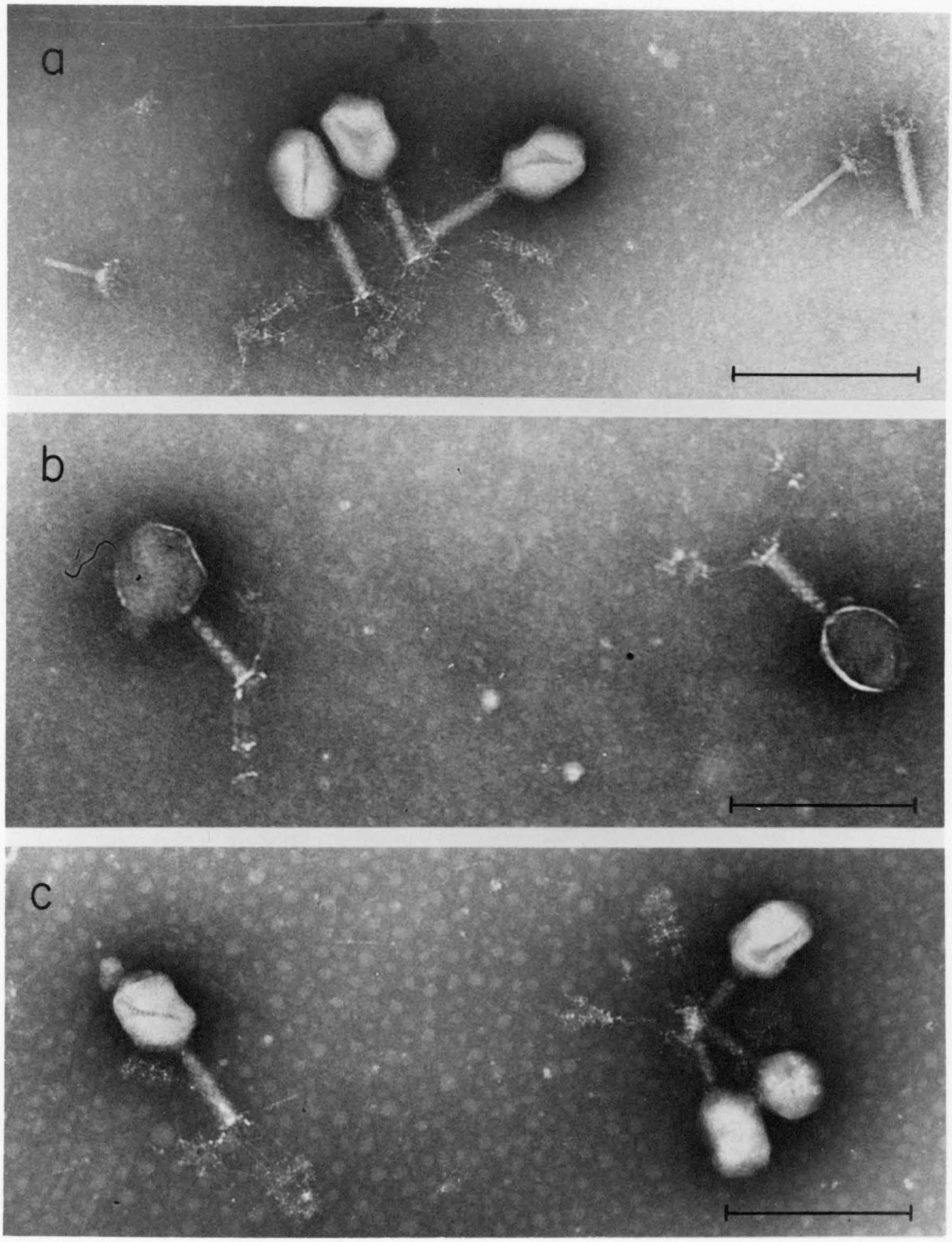
hy3 blocks all of the activity of AS1 although the left end of its gene 37 comes from T2. This result might also be explained in two ways, lack of T4-specific antigens in this region or redundancy of its antigens with antigens in the rest of P37. Although it has not been possible to test the two possibilities for this region, it seems likely, since T2 and T4 are homologous in this region (Beckendorf et al., 1972), that adsorption of the T4 BC' serum with T2 removed all antibodies specific for this region; that is, this region probably lacks T4-specific antigens.

(c) Electron microscopy of tail fiber:antibody complexes

The preceding section demonstrates that most parts of $P37^4$ determine T4 specific antigens and that a T4 segment within a largely T2 P37 still specifies T4 antigens. The T2 adsorbed serum AS1 can be used as a specific stain so that such a segment can be visualized by electron microscopy. In this way the transition point (TP) between T2 and T4 protein in the half fiber can be determined. By comparing these TP's with the XOP's established by genetic and physical mapping of gene 37, we have been able to determine the topology of P37 in the half fiber and to gain some understanding of its conformation.

(i) Binding of anti-T4 BC' serum to T2 and T4. When anti-T4 BC' serum was reacted with T4, the entire distal half of the tail fiber was covered with antibodies (Plate 1a). Similar results were obtained by Yanagida & Ahmad-Zadeh (1970) using whole anti-T4 serum which had been adsorbed with a 37^- lysate.

Plate 1. Attachment of antibodies to the tail fibers of T4 and T2. Phage placed on grids, incubated with antiserum, fixed and stained as described in Materials and Methods. The scale in each frame represents 500 Å. a. T4 with anti T4 BC' serum. b. T2 with anti T4 BC' serum. c. T4 with AS1..

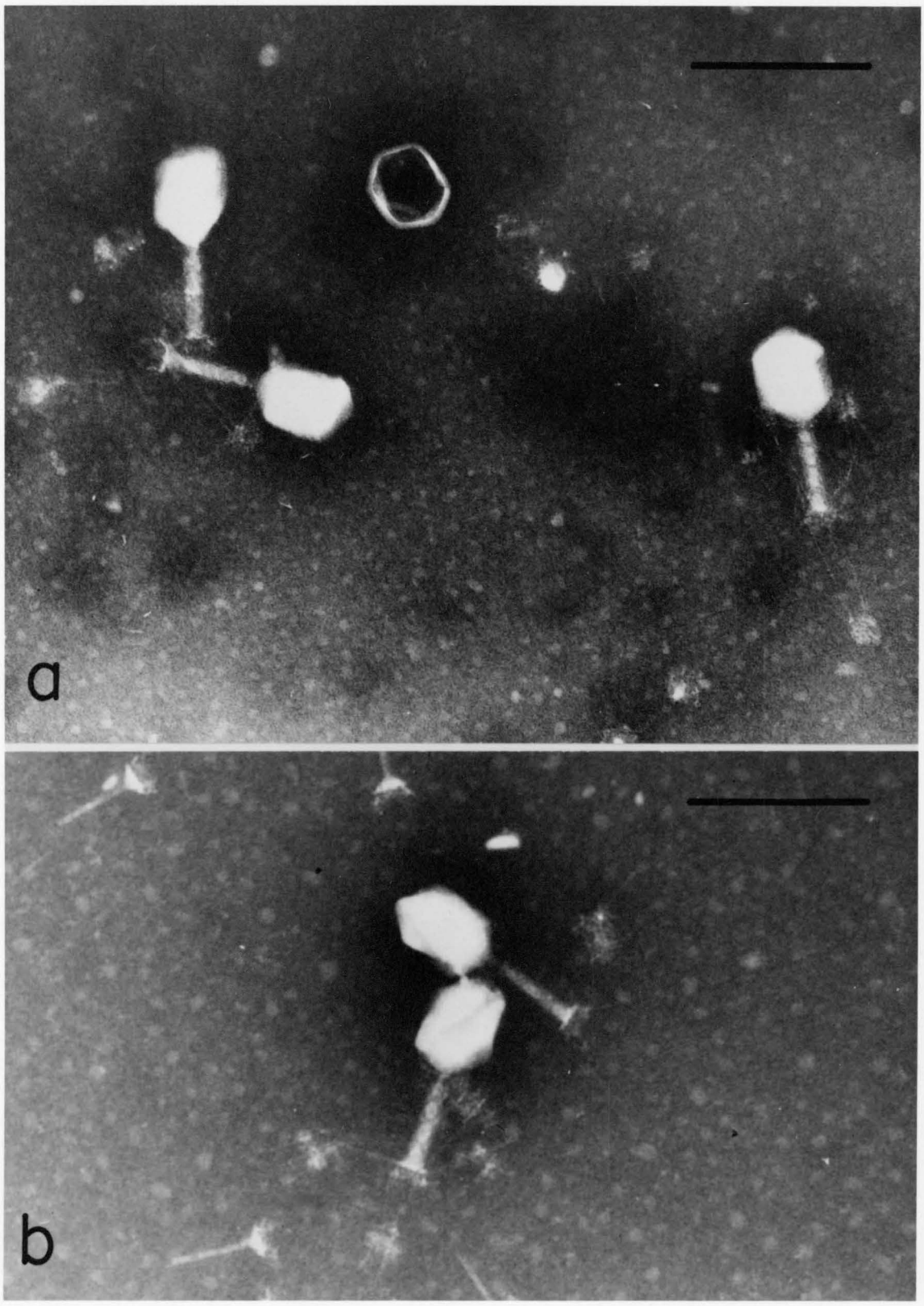

a
b
c

When T2 was incubated with the anti-T4 BC' serum for 4-6 hours, much of the proximal part of the distal half fiber was covered with antibodies. However, when the incubation was only 2 hours the antibody was clustered at two narrow zones, from 150 to 250 Å and from 490 to 570 Å from the joint between the half fibers (Plate 1b). Under comparable conditions T4 fibers are completely covered. These results are consistent with the fact that T2 blocks only 8% of this serum and suggest that most of the crossreacting antibodies are directed against two small segments of the T2 distal half fiber.

(ii) Binding of AS1 to T4. When T4 is reacted with AS1 the fibers are densely coated with antibodies from a point 170 Å away from the joint to their distal ends (Plate 1c). Sometimes a small amount of antibody is visible right next to the joint. Thus most of the antibodies in the anti-T4 BC' serum which reacted with the proximal 170 Å were removed by adsorption with T2. The antibody sometimes visible near the joint probably represents the anti-B antibodies which remain in AS1.

(iii) Binding of AS1 to hybrid phage. Hybrid phage hy5 has the left end of its gene 37 from T4 and the right end from T2. From genetic and physical mapping of gene 37 (Beckendorf et al., 1972) the intersection between T4 and T2 DNA occurs between .35 and .48. When reacted with AS1 only part of the distal half fiber of hy5 is coated with antibody (Plate 2a). The 170 Å nearest the joint of the fiber is uncoated, as it is in T4, and the terminal 310 Å is also uncoated. hy54, on the other hand, has T2 DNA at the left and T4 DNA at the right end of gene 37 with an XOP between .50 and .81. As shown in Plate 2b,

Plate 2. Attachment of antibodies to the tail fibers of T2:T4 hybrid phages. Phage were placed on grids, incubated with antiserum, fixed and stained as described in Materials and Methods. The scale in each frame represents 500 Å. a. hy 54 with AS1. b. hy 5 with AS1.


a
b

hy54 when reacted with AS1 has antibodies complexed with only the distal end of the fiber. These two results indicate that the protein coded by the left end of gene 37 is located near the joint between the two half fibers, and the protein coded by the right end is located near the distal tip of the BC' half fiber. Since gene 37 is translated from left to right, the amino terminus of P37 is near the joint and the carboxy terminus is near the tip of the BC' half fiber.

P37 must become considerably condensed during assembly, since in an extended or alpha-helical form it would be three or more times as long as the C half fiber (Ward et al., 1970; Cummings et al., 1970). It is possible to envision any number of configurations having the N terminus at one end and the C terminus at the other. To investigate the topology of P37 in the half fiber we have determined the position of AS1 antibodies on the tail fibers of six hybrid phages (Table 3). From these measurements we have calculated the transition points (TP's) between T2 and T4 protein in the assembled fiber, and compared them with the XOP's established by mapping of gene 37.

Two types of corrections have been applied to the measurements of antibody coated fibers in determining TP position. First, since BC' half fibers which had antibodies on their tips were about 100 Å longer than those without and since antibody molecules are about 100 Å long (Feinstein & Rowe, 1965), this distance was subtracted from the length of those tail fibers with antibodies on their tips. This correction aligned the TP's of reciprocal hybrids, one of which had T4 protein at the distal end of its tail fiber and one of which had T2 protein at this end. Second, since the C half fiber, which contains only P37, is

Table 3

Location of AS1 Antibodies Attached to Tail Fibers

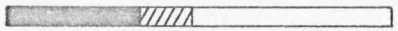
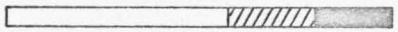
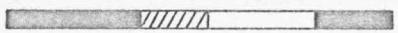
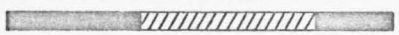
Phage		Joint to Ab	Ab^1	Ab^1 to Ab^2	Ab^2	Ab to tip	BC'
T4D		170±30(5)	610±50(9)				790±75(5)
hy3		180±50(10)	590±95(20)				790±60(10)
hy5		170±45(9)	200±52(15)			320±70(23)	670±40(12)
hy16a		170±45(8)	270±50(30)			280±60(20)	720±40(6)
hy54		450±50(9)	330±25(17)				780±50(9)
hy172		170±40(10)	200±70(6)	170±30(6)	260±50(6)		790±60(10)
hy174		170±40(11)	130±30(8)	170±30(8)	310±65(6)		790±30(9)

Table 3 (continued)

Location of AS1 antibodies attached to tail fibers. The bars in column 2 depict the composition of gene 37 (see Table 2). The measured lengths are given in Å ± the standard deviation. The numbers in parentheses are the number of fibers measured. All values were normalized by assuming that the A half fibers measured on the same grids were 690 Å long (Ward et al., 1970). Column 3 gives the distances from the joint between the two half fibers to the first antibody cluster. Column 4 gives the size of the first antibody cluster, column 5 the distance between the first and second clusters, and column 6 the size of the second cluster. Column 7 gives the distance from the last antibody cluster to the tip of the fiber, and column 8 gives the total length of the BC' fiber with its attached antibodies.

130 Å shorter than the finished BC' half fiber which also contains P36 and probably P35 (Eiserling & Dickson, 1972), and since the B antigen, whose appearance is controlled by P36 (Edgar & Lielausis, 1964), is located at the joint between the A and BC' half fibers (Yanagida & Ahmad-Zadeh, 1970), the proximal 130 Å of the half fiber was assumed not to be P37 and was subtracted from all fiber lengths. This correction aligned the TP of hy3 which is very near the amino terminus of the protein with its genetically determined XOP. The values of the TP's obtained after these corrections are listed in Table 4 along with the XOP's.

The XOP's can be arranged in a linear sequence of four nonoverlapping groups, thereby dividing the gene into 5 nonoverlapping parts (Table 5). By comparing this sequence with that of the TP's we can determine whether or not the corresponding 5 parts of P37 are arranged in a linear order in the assembled fiber. Table 5 shows that the TP order is in complete agreement with the XOP order. We conclude that P37 in the assembled tail fiber is colinear with the polypeptide chain and with gene 37.

(iv) Structure of the tip of the tail fiber. Besides establishing the linearity of P37 in the half fiber, these results can be used to suggest differences along the fiber in the conformation of P37. If the conformation were the same throughout the fiber, each Å along the fiber would contain the same number of amino acids or the same fraction of P37. Given such a P37 structure, a plot of TP's (as Å between the proximal end of P37 in the fiber and the transition from T2 to T4 protein) against the corresponding XOP's (as the fraction of P37 to the left of

Table 4

Transition points of hybrid phages

Hybrid	XOP	Transition points	
		Proximal end to TP	560-(TP to distal end)
hy3	.08-.13	50 ± 50	70 ± 95
hy5	.35-.48	240 ± 52	240 ± 70
hy16a	.58-.68	310 ± 50	280 ± 60
hy54	.58-.80	320 ± 50	330 ± 25
hy172	.35-.52	240 ± 70	230 ± 60
	.80	410 ± 76	400 ± 50
hy174	.35-.52	170 ± 30	180 ± 71
	.52-.80	340 ± 43	350 ± 65

XOP's were calculated as for Table 2. The TP's were determined from the data in Table 3 after two corrections were made (see text). (1) When antibodies coated the distal end of a tail fiber, 100 Å were subtracted from its distal end. (2) 130 Å were subtracted from the proximal end of all measured fibers. After these corrections the position of each TP was determined twice, as indicated at the tops of the two columns. The TP positions are expressed as Å away from the proximal end of P37 ± the standard deviation.

Table 5

Linearity of P37 in BC'

ORDER OF XOP's
hy3 < hyl74-A, hyl72-A, hy5 < hyl6a, hy54, hyl74-B < hyl72-B
ORDER OF TP's
hy3 < hyl74-A < hyl72-A, hy5 < hyl6a, hy54, hyl74-B < hyl72-B

hyl72-A represents the proximal and hyl72-B represents the distal XOP or TP in hyl72. The same notation is used for hyl74.

the same transition) would yield a straight line. When such a plot is constructed, most of the points lie above this line. Although there are large uncertainties in the positions of all of the points, these data suggest that P37 is in a relatively more extended configuration near the distal end of the fiber (dotted line, Fig. 4). Since P37 is the only protein in this end of the tail fiber, if it were in an extended form, the diameter of the fiber would be less than in the rest of the fiber. This prediction prompted a survey of available electron micrographs of T4. In fact high resolution micrographs often show near the distal tip of the tail fiber a thinner portion which in some preparations is bent at an angle relative to the rest of the fiber (Plate 5). This thin portion is not the result of a staining artifact near the ends of all fibers since isolated C, BC, and BC' half fibers often show a single thin end very similar to that seen on phage or isolated whole fibers (see Ward et al., 1970, Plate III). A number of electron micrographs which showed this thin portion were kindly loaned to me by Dr. R. C. Williams. The thin parts of 33 fibers were measured and found to be 180 ± 30 Å long.

4. Discussion

(a) Antigenic specificities on the BC' half fiber

Serum blocking experiments with the phages which carry a T2:T4 hybrid gene 37 allow the antigens determined by gene 37 to be divided into at least four and maybe five classes with different specificities (see Table 6). Class four is deduced from the fact that when T2 is reacted with anti-T4 BC' serum, antibodies are attached to the fibers in a region corresponding to .58-.80 of $P37^4$ (Plate 1b). This region

Fig. 4. Comparison of XOP's and TP's. The XOP's are expressed as fraction of gene 37 and TP's as Å away from the proximal end of P37 (see Table 4). The solid line is that expected if the structure of P37 were constant throughout the fiber. The dotted line is suggested by the data (see text).

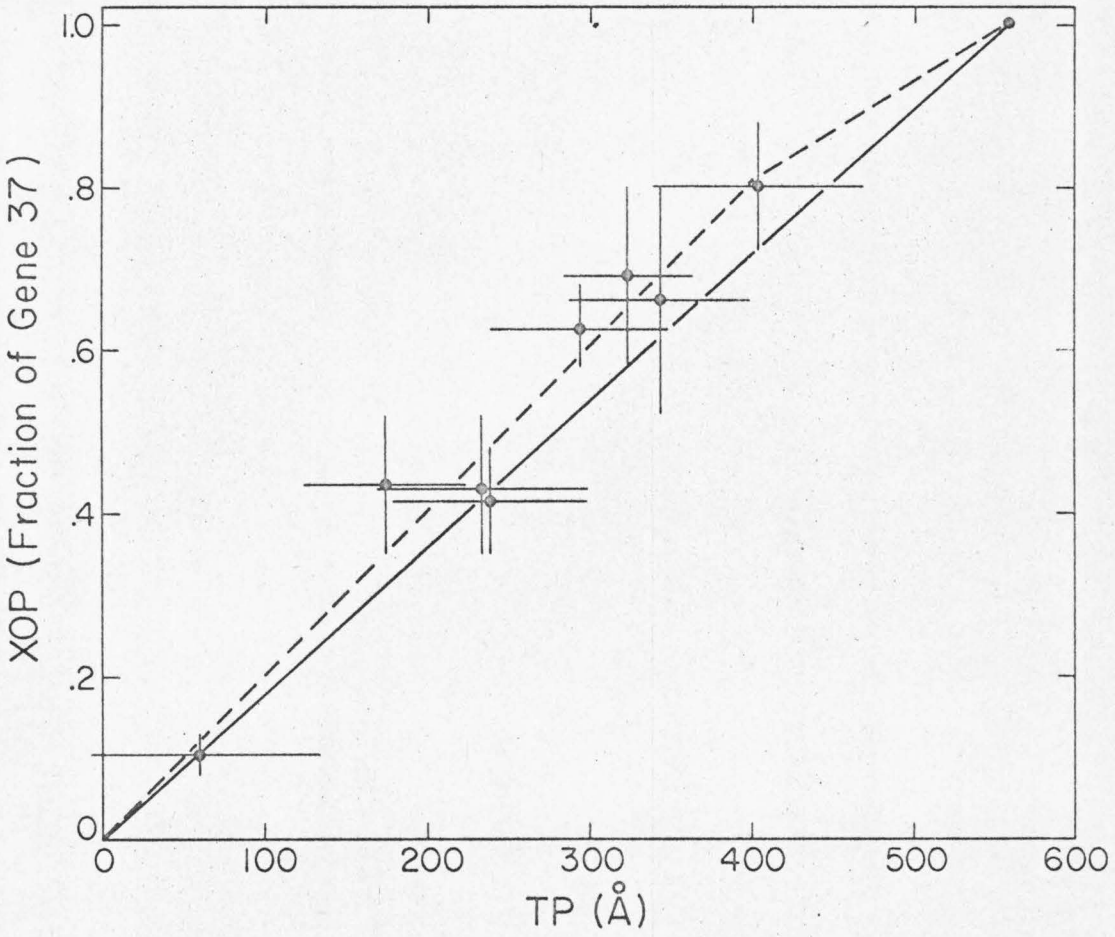

XOP (Fraction of Gene 37)
1.0
.8
.6
.4
.2
0
0
100
200
300
400
500
600
TP (Å)

Plate 3. T4D negatively stained with phosphotungstic acid. Electron micrograph taken by R. C. Williams under conditions of minimal illumination by the electron beam in order to preserve detail in the specimen (Williams and Fisher, 1970). Arrows indicate the thin portions of the distal half fiber discussed in the text.

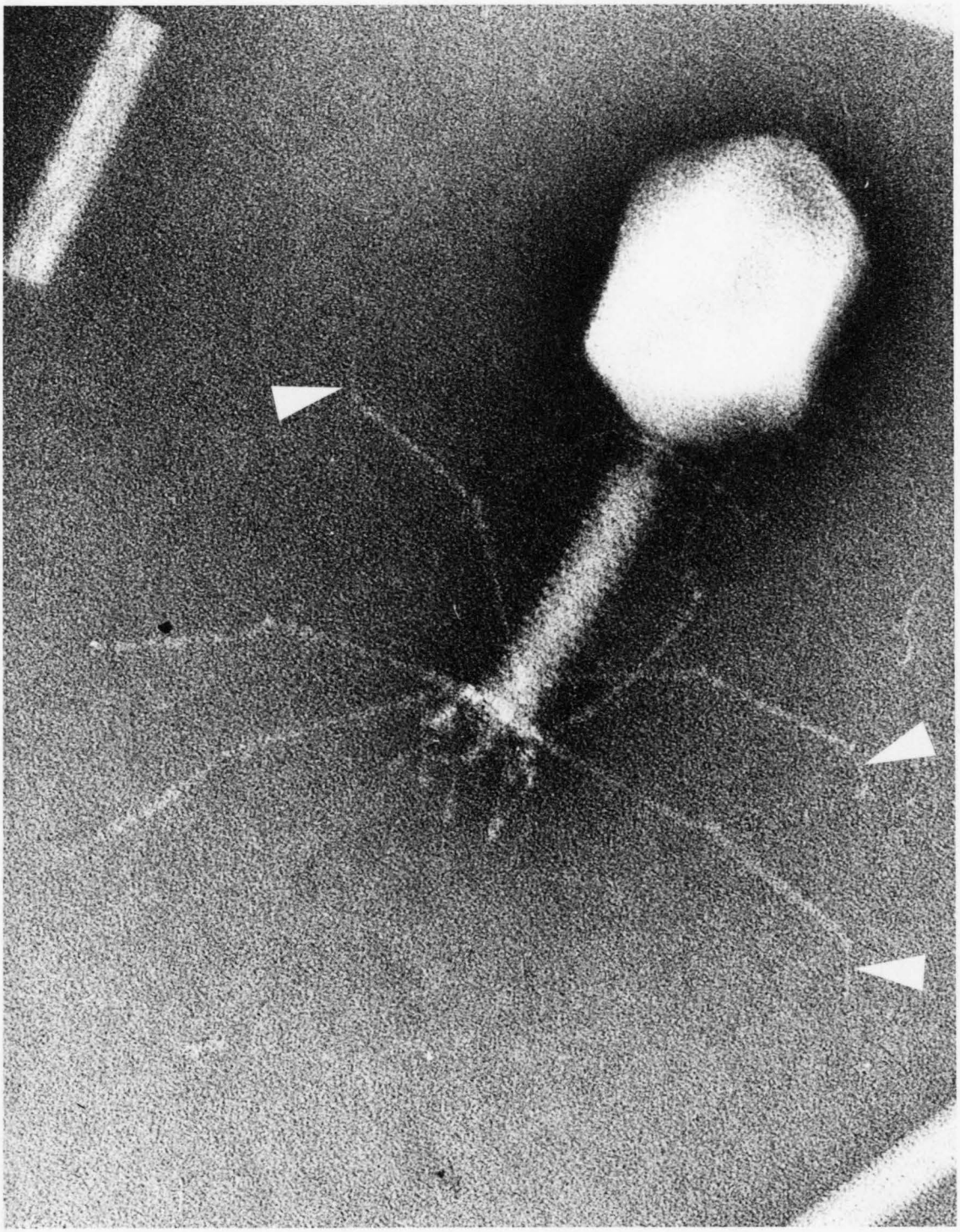


Table 6

Antigens specified by gene 37

Class	Location in gene 37	Specificity
1	0 - XOP^3	Common with T2
2	XOP^3 - XOP^5	Unique
3	XOP^5 - .58	Shared with .58-.80
4	.58-.80	Common with T2
5	.80-1.0	Unique

XOP^3 and XOP^5 indicate the XOP's of hy3 and hy5 respectively. Classes 1, 2, 3, 5 were deduced from the serum blocking results with AS1 (Table 2). Class 4, deduced from interaction of anti-T4 BC' serum with T2 (Plate 2), may share all or part of its specificity with Class 1.

of T4 was previously shown to be homologous to T2 (Beckendorf et al., 1972), so some cross reactivity was expected.

Do genes other than 37 specify some of these antigens? Since genes 57 and 38 are involved in the assembly of the C half fiber, they are obvious candidates for the role.

P57 is also involved in the assembly of the A half fiber which carries no antigens which cross react with the C or BC' half fibers. Therefore, P57 probably does not specify any antigens. It still might modify amino acids of P37 and in the process create antigens, but the specificity of these antigens would be determined by the amino acid sequence of P37, not by P57.

We cannot tell whether P38 adds antigens to the C half fiber. Since the host range region of T4 gene 37 is always associated with T4 gene 38, the function of P38 might well be to modify P37 thereby allowing its attachment to polysaccharides in the cell wall.

The possibility that an undiscovered tail fiber gene or genes might control the specificity of the C antigens seems remote. Numerous searches for additional essential T4 genes (Edgar & Lielausis, unpublished) or specifically for essential tail fiber genes (Beckendorf & Lielausis, unpublished) have proven fruitless. Isolation of mutants in T2 and T6 have also turned up no new tail fiber genes (Russell, 1967).

Since there are at least four distinct subsets of the C antigen and only one gene besides gene 37 which might be contributing to them, the amino acid sequence of P37 must determine most of the antigens. It was shown above that some antigenic sites are present in more than one part of the gene product. All of the antigens in 22% of the gene

product are repeated and are probably the only antigens in another 10-20%. These observations suggest that there are some repeating amino acid sequences in P37 and perhaps that the fiber has some regular, periodic structure over part of its length.

(b) Structure of the assembled BC' half fiber

The results in section 3(c)(iii) establish that P37 is oriented linearly with its amino terminus near the joint between the two half fibers and its carboxyl terminus near the distal tip of the BC' half fiber. An alternative model which proposed that P37 was folded back on itself twice to yield a three-stranded structure (Cummings et al., 1970) is clearly ruled out.

The C half fiber contains two copies of P37 and no other proteins (Eiserling & Dickson, 1972). It is an asymmetrical structure, with one thin end which eventually becomes the distal tip of the finished fiber. This asymmetry, as well as the results obtained from the electron microscopic observations of antibodies attached to hybrid phage, shows that the two copies of P37 are oriented parallel to each other in the assembled fiber.

The final .20 at the carboxyl end of P37 determines the host range of the phage, interacts with P38, and probably interacts with the bacterial surface (Beckendorf et al., 1972). This region contains about 190 amino acids and from the position of the distal XOP in hy172 occupies the distal 160 $\pm$ 50 Å of the tail fiber. Thus this functionally specialized region corresponds quite closely with the structurally specialized thin tip which occupies the distal 180 $\pm$ 30 Å. Assuming the structure is constant for this 180 Å, the thin tip would contain

about 210 amino acids. The thick part of P37 in the fiber is then about 380 Å long and contains about 740 amino acids. By dividing the length of a region of the tail fiber by the number of amino acids it contains, a parameter d, the distance, measured along the fiber axis, from one amino acid to the next, can be calculated. In Table 7 values of d for the thin and thick parts of P37 values are compared with similar values for several protein secondary structures. As can be seen, only the γ helix is compact enough to fit the host range region. None of the structures is appropriate for the proximal part of P37. The γ helix was proposed by Pauling & Corey (1951) as a possible protein conformation, but if it existed it would be extremely unstable (Ramakrishnan & Ramachandran, 1965). Since tail fibers are stable to a number of denaturing conditions it seems unlikely that the tip is made up of γ helix. Therefore, P37 in the C half fiber, which consists of just two polypeptide chains oriented linearly and parallel to one another, does not seem to assume any of the simple secondary structures which have been proposed.

The data presented in this paper and the accompanying one (Beckendorf et al., 1972) can be summarized in a model of the distal half fiber (Fig. 5).

Several indirect lines of evidence suggest that P36 occupies the proximal 130 Å of the half fiber. It is responsible for the production of the B antigen (Edgar & Lielausis, 1965), which is localized near the joint between the half fibers (Yanagida & Ahmad-Zadeh, 1970). P36 is incorporated into the half fiber (Eiserling & Dickson, 1972) and causes the elongation of the 560 Å C half fiber to the 690 Å BC

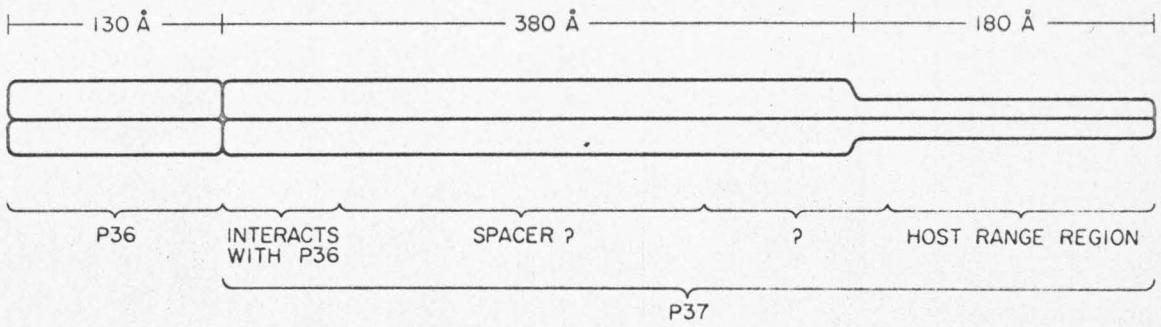
Table 7

Comparison of P37 and known polypeptide structure

	d
P37 thick	.51
P37 thin	.86
γ helix	.98
π helix	1.15
α helix	1.50
collagen helix	2.95
β structure	3.45

For the two parts of P37, d is the length in Å divided by the number of amino acids in that part [based on a molecular weight of 105,000 for P37 (Beckendorf et al., 1972)]. For the other structures, d is the rise in Å along the helix axis per amino acid. The values of d for these structures are from Ramakrishnan & Ramachandran (1965).

Fig. 5. A model of the BC' half fiber. The distal end of the half fiber is to the right. Each enclosed area represents a polypeptide chain. The question mark following "spacer" indicates that this is a hypothetical function for this region. Two possibilities for the function of the adjacent region are proposed in the text.


130 Å
380 Å
180 Å
P36
INTERACTS WITH P36
SPACER ?
?
HOST RANGE REGION
P37

half fiber (Ward et al., 1970). Since both the C and BC fibers have a similar thin end which eventually becomes the distal end of the fiber, P36 must not be added to the distal end. If P36 increased the length of the thick part of the half fiber by intertwining with P37, the P37 in this part of the fiber would be extended relative to the C half fiber and the proximal end of P37 would be closer than 130 Å to the joint. The position of the TP in hy3 indicates that P37 is not in an extended form distal to this TP. Therefore, P36 might be intertwined with the proximal 0.10 of P37 or it might be separate as shown.

An important feature of the model is that it shows several functional regions of P37. As discussed in the accompanying paper, these can be correlated with regions which differ in their amount of homology between T2 and T4. At the proximal end of P37 is a region which must interact with P36. Some ts mutations of this region apparently prevent this interaction. Cells infected with these mutants produce normal amounts of C antigen but no B antigen (Edgar & Lielausis, 1965). The T4 DNA which codes for this region of P37 is homologous with the corresponding T2 DNA and the regions cross react serologically as might be expected if they have to interact with similar sites on $P36^2$ and $P36^4$. In agreement with this idea is the finding that $P36^2$ and $P36^4$ are functionally interchangeable (Russell, 1967).

The adjacent region has an apparently uniform structure for over 300 Å. The serum blocking results suggest that the structure is repetitive and periodic. It is coded for by DNA which is largely nonhomologous between T2 and T4 and yet is interchangeable in part or in toto between the two phages. These results suggest that the function

of this region is not dependent on a specific amino acid sequence and thus a large number of functionally neutral mutations have occurred since the divergence of T2 and T4. This region may be thought of as a structural spacer between two specifically functioning regions.

Adjacent to the spacer region is a short region whose DNA is homologous between T2 and T4 and whose antigens cross react. That T2:T4 homology has been retained may indicate that this region has a more specialized function than the adjacent spacer region. The change in diameter of the fiber occurs either in this region or at its distal end. The point at which the change occurs is not as rigid as the rest of the fiber since a bend is sometimes seen at this point in electron micrographs. It could be that this region is still homologous in T2 and T4 because a particular sequence is required for the change in structure. Another possible function for this region is interaction of BC' with the phage head. Both kinetic (Terzaghi, 1971) and serological (J. Latta & W. B. Wood, personal communication) evidence suggests that BC' interacts with the head during attachment of tail fibers to fiberless particles. In assembled phage only this short homologous segment and the host range region are far enough away from the baseplate to interact with the head.

At the tip of the fiber is the structurally specialized host range region with a smaller diameter than the rest of the fiber. As might be expected from the difference in the bacterial receptors for T2 and T4, this region has changed most during the evolutionary divergence of the two phages. The T2 and T4 DNA sequences coding for this region

are completely nonhomologous and specify polypeptide sequences of different length.

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General Discussion

The results in parts I and II of this thesis extend our knowledge of the structure of the distal half fiber, which is the phage component in the interaction between T4 and the bacterial surface. To understand this interaction, we need to know the structure of the bacterial component and to have more detailed information about tail fiber structure. The first part of the discussion below describes a number of experiments aimed at understanding the bacterial receptor for T4. The second part proposes a detailed model for the structure of the distal half fiber and discusses experiments suggested by this model.

Very little work has been done on structure of the bacterial receptor for T4 since Weidel and coworkers demonstrated that lipopolysaccharide (LPS) extracted from sensitive cells is able to inactivate T4 (Weidel, 1958). These workers also showed that LPS from a resistant cell is unable to inactivate T4 and that, while LPS from sensitive cells contains glucose and galactose, the LPS from resistant cells contains no detectable hexose. Thus it appears that T4 requires hexose or some other constituent which was present in the wild type, but not the mutant, LPS. Wilson, Luftig, and Wood (1970) confirmed that LPS inactivates T4 and showed that it interacts with the tip of the tail fiber.

To determine more precisely which constituents of the LPS are important for T4 attachment, I have isolated a large number of T4-resistant strains of *E. coli* K12. Those which are sensitive to

T4 host range (h) mutants have been selected for further study because they were expected to have small changes in their LPS. Analysis of the polysaccharide composition of the LPS from these strains would show how the LPS can be changed to prevent T4 adsorption. It might then be possible to deduce which parts of the LPS are essential for T4 attachment.

These resistant bacteria can be divided into at least six classes by their sensitivity to T4 h mutants and other bacteriophages. The alterations in the LPS of some of the bacteria change the specificity of tail fiber attachment while others apparently change the rate of attachment. These results suggest that there is considerable variety in the T4-receptors of these strains. Therefore, chemical analysis of their LPS should help to characterize the structure of the receptor.

For any meaningful understanding of adsorption, the structure of the distal half fiber must be known in detail. The model presented in the discussion of part II accounts for all of the data which has been presented, but it is still quite incomplete. It does not specify the secondary structure of P37 nor any interactions between the two copies of P37. Furthermore it does not specify the location of the third protein in the half fiber, P35. For these reasons I have constructed a more detailed and more speculative model of the distal tail fiber. This model makes some predictions which should be experimentally testable.

As pointed out above, the thick part of P37 and probably the

thin part as well are too condensed to fit any known helical protein structure. I propose that the two copies of P37 in the thick part of the half fiber are coiled into a single, two-stranded helix so that they form a hollow cylinder. If this cylinder were cut down one side and laid flat, the polypeptide chains would be arranged as in a parallel β-pleated sheet (Pauling and Corey, 1951) with the two copies of P37 alternating as the parallel polypeptide chains. Since the spacing between two chains in a β-sheet is about 4.5 Å and there are two copies of P37 in the tail fiber, the distance between turns for one of the chains would be about 9 Å. The rise per residue for this part of the fiber is 0.5 Å/aa so there would be 18 aa/turn of the helix. The spacing between amino acids on one chain is 3.5 Å for β-structures so the diameter of the helix would be about 20 Å. Since the amino acid side chains will extend about 2.5 Å on either side of the backbone, the diameter of the fiber would be about 25 Å and the diameter of the inner hole would be about 15 Å.

A similar structure can be constructed for the thin tip of the fiber. It has 10 aa per turn, an outer diameter of 16 Å and an inner diameter of 6 Å.

The molecular weight of P36 has been measured as 24,000 (Eiserling and Dickson, 1972). If P36 occupies the proximal 130 Å of the half fiber (see part II, discussion), then it would have a rise per residue of 0.6 Å/aa. With a structure similar to P37 it would have 15 aa per turn of helix, an outer diameter of 22 Å and an inner diameter of 12 Å, essentially the same structure as the thick part of P37.

This model predicts that there is a hole in the center of P36 and at least the proximal part of P37. A third protein, probably P35, has been found in this half fiber (Eiserling and Dickson, 1972). Unlike P36 and P37 it is present in only one copy in the tail fiber and does not add any antigens to the fiber. A possible explanation for these facts is that P35 fills the hole in the proximal part of the half fiber. This hole is just about large enough in diameter to accept a single copy of an α-helical protein. In filling this hole P35 might stabilize the complex of P36 and P37. This idea would explain why purified C and BC half fiber intermediates are inactive, while BC' half fibers are active (Ward et al., 1970). This model might also explain how P35 prepares the distal half fiber for attachment to the proximal one. The length of the thick part of the half fiber and presumably of the hole in its center is about 510 Å. An α-helical protein the size of P35 (40,000 daltons, Eiserling and Dickson, 1972) would be about 550 Å long. Thus P35 might project proximally to the proximal end of P36. If there were a corresponding hole in the proximal half fiber, the end of P35 might fit into it, thereby making the rigid joint between the two half fibers (Brenner et al., 1959; Ward et al., 1970).

This model might also explain the instability of free P35 (King and Wood, 1969). A protein in close contact with other proteins on all sides would probably be very hydrophobic, and if free in solution, would probably aggregate with itself or other hydrophobic molecules.

The model suggests several directions for future research. The

low angle X-ray patterns of aligned half fibers should show a high degree of order and strong periodic reflections indicating the periodicity of the helix. Furthermore, the pattern should differ in a regular way in the absence of P35.

Analysis of the sequence of P37 might well yield information about the structure of the fiber. A number of methods have been developed for predicting structure from primary sequence (Venkatachalam and Ramachandran, 1969). Although the methods have not been developed for a parallel β-structure, similar approaches might yield useful information. The observation of repeated antigens in the thick part of P37 suggests repeated amino acid sequences which might be valuable in predicting structure. The model predicts a similar structure for P36 and P37. This similarity might be evident in their sequences.

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A Recombination Gradient in Bacteriophage T4 Gene 34

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INTRODUCTION

Analysis of recombination within T4 genes 34 and 35, using a standard T4 mapping function to relate recombination and physical distance, suggests that these genes are quite large relative to other T4 genes (Stahl, Edgar and Steinberg, 1964). However, the size of the polypeptide produced by gene 34 (King and Laemmli, 1972; Ward and Dickson, 1972) and a physical map of the T4 chromosome (Mosig, 1968) indicate that the actual size of genes 34 and 35 is well below the genetic estimate. The inadequacy of the mapping function to determine the physical size of these genes suggests a recombinational anomaly in this region of the T4 genome. This anomaly apparently does not exist in the very closely related phage T2. T2 genes 34 and 35, which can substitute for the corresponding T4 genes, appear as normal sized genes on the T2 genetic map (Russell, 1967).

To determine whether the high recombination in T4 gene 34 is due to one or a few recombinational hot spots or to a homogeneous increase in recombination throughout the gene, we have compared physical and genetic maps of T4 gene 34 and T2 gene 34. Since polypeptide chain termination occurs at the amber codon under nonpermissive conditions, an amber mutation can be positioned physically within a gene by determining the relative sizes of the wild type product and the amber fragment using the technique of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS). Because the product of gene 34 (P34) is the largest polypeptide in the phage, its amber fragments are relatively easy to identify. If there is a homogeneous increase in recombination throughout the gene, the genetic and physical positions

of the amber mutations will agree. In contrast, if there are recombinational hot spots, the genetic and physical positions of the amber mutations will differ, and in the region near a hot spot the genetic map will be distorted relative to the physical map.

MATERIALS AND METHODS

Phage and bacterial strains. Phage strains derived from the wild type T4D were obtained from the collection of R. S. Edgar and have been described elsewhere (Epstein et al., 1963; Edgar and Lielausis, 1965; Wilson and Kells, 1972). Phage strains derived from wild type T2L were obtained from the collection of R. L. Russell.

Escherichia coli strain CR63 was used as permissive host for T4 amber (am) mutants, strain CR63$r_6^-r_{24}^-$ as permissive host for T2 am mutants (Georgopoulous and Revel, 1971), and strain S/6/5 as nonpermissive host for both T2 and T4 am mutants. Strain Bb, which is also nonpermissive for am mutants, was used as host in the preparation of all lysates (Wilson et al., 1970).

Media. H broth used for phage and bacterial growth, and EHA top and bottom agar used for plating assays, were prepared as described by Steinberg and Edgar (1962). Dilution buffer was prepared as described by King (1968). Two different minimal growth media were used for preparation of radioactively labeled infected-cell lysates. Minimal medium A, contained per liter 7 gm Na_2HPO_4, 3 gm $KH_2 \cdot PO_4$, 6 gm NaCl, 1 gm NH_4Cl, 0.12 gm $MgSO_4$, 0.01 gm $CaCl_2$, and 4 gm glucose. Minimal medium B is identical to minimal medium A except that it has only 1 gm NaCl and no $CaCl_2$ added per liter. No differences were detected in results obtained with the two minimal media.

^{14}C-labeled lysates. Strain Bb was grown to 5 x 10^7 cells/ml in minimal medium A or B, collected by centrifugation, and resuspended in minimal medium at 2-4 x 10^8 cells/ml. One ml aliquots of this suspension were warmed to 37°, infected with phage at a multiplicity of 4-10, and aerated by bubbling or by agitation on a rotary shaker. 14 or 15 min after infection 2-4 µC of a uniformly-labeled ^{14}C amino acid mixture (Schwarz-Mann) were added. At 45 min after infection the samples were treated one of two ways. In the first method 2 drops of $CHCl_3$ were added to each sample and the samples were thoroughly mixed. The lysates were then frozen in dry ice-ethanol and thawed. DNase (2.5 µg/ml) and lysozyme (10 µg/ml) were added and the lysates were incubated at 37° for 15 min. In the second method the lysates were added at 45 min to an equal volume of cold 10% TCA. All lysates were dialyzed against 0.065 M Tris-HCl, pH 6.8, 1.0% SDS. When the lysates are analyzed on discontinuous polyacrylamide gels containing SDS, the two methods give different relative amounts for some of the bands but the relative rates of migration of the bands are identical.

Gel electrophoresis and autoradiography. Procedures for the preparation and running of discontinuous polyacrylamide gels containing SDS were as described by Laemmli (1970). Sample preparation, staining, destaining and autoradiography of the gels were as described by Wilson and Kells (1972). The autoradiographs were traced at 2- to 20-fold magnification with a Joyce-Loebl microdensitometer and the migrations of particular bands relative to the dye marker or to another reference band were determined from the tracings. The molecular weights of the amber fragments were determined by comparing their migration with that

of a set of standard proteins (Fig. 1). Although the standard proteins run at the same relative rates in standard and discontinuous SDS gels (i.e., a plot of mobility against the log of molecular weight yields a straight line with both systems), we find that several of the phage proteins do not. This discrepancy between the two gel systems has also been noticed by R. C. Dickson (personal communication). Perhaps because of this anomolous behavior or because of other differences in procedure the molecular weight values we have determined from discontinuous SDS gels differ from those previously reported. Specifically we have determined the molecular weights of T4 P34 and P37 to be 130,000 daltons and 105,000 daltons respectively whereas the previously published figures were 150,000 daltons for P34 and about 120,000 daltons for P37 (Ward and Dickson, 1972; King and Laemmli, 1972). We have recently obtained independent evidence that the value of 105,000 daltons for P37 is closer to the actual value than the previously determined 120,000 daltons (Beckendorf, Kim and Lielausis, 1972).

Phage crosses. Standard phage crosses were done as described by Wilson and Kells (1972). CR63 was used as host for T4 crosses. CR63$r_6^-r_{24}^-$ was used as host for T2 crosses. T4 crosses were done at 30°, T2 crosses at 25°.

RESULTS

Genetic maps of gene 34 in T2 and T4 were constructed from the results of two factor crosses and are presented in Figs. 2 and 3. Only four am mutants were available in T2 gene 34. In agreement with previous results (Stahl et al., 1964; Russell, 1967) the total amount of recombination in T4 gene 34 (42%) is greater than that in T2 gene 34 (16%).

Fig. 1. Mobility on discontinuous SDS gels of proteins used as molecular weight standards. Mobilities are relative to bromphenol blue (Rf = migration of protein/migration of bromphenol blue). The standard proteins in order of decreasing molecular weight are γ-globulin, β-galactosidase, phosphorylase a monomer, serum albumin, catalase, H-chain γ-globulin, ovalbumin, L chain γ-globulin. The sources and molecular weights of these proteins are in Weber and Osborn (1969).

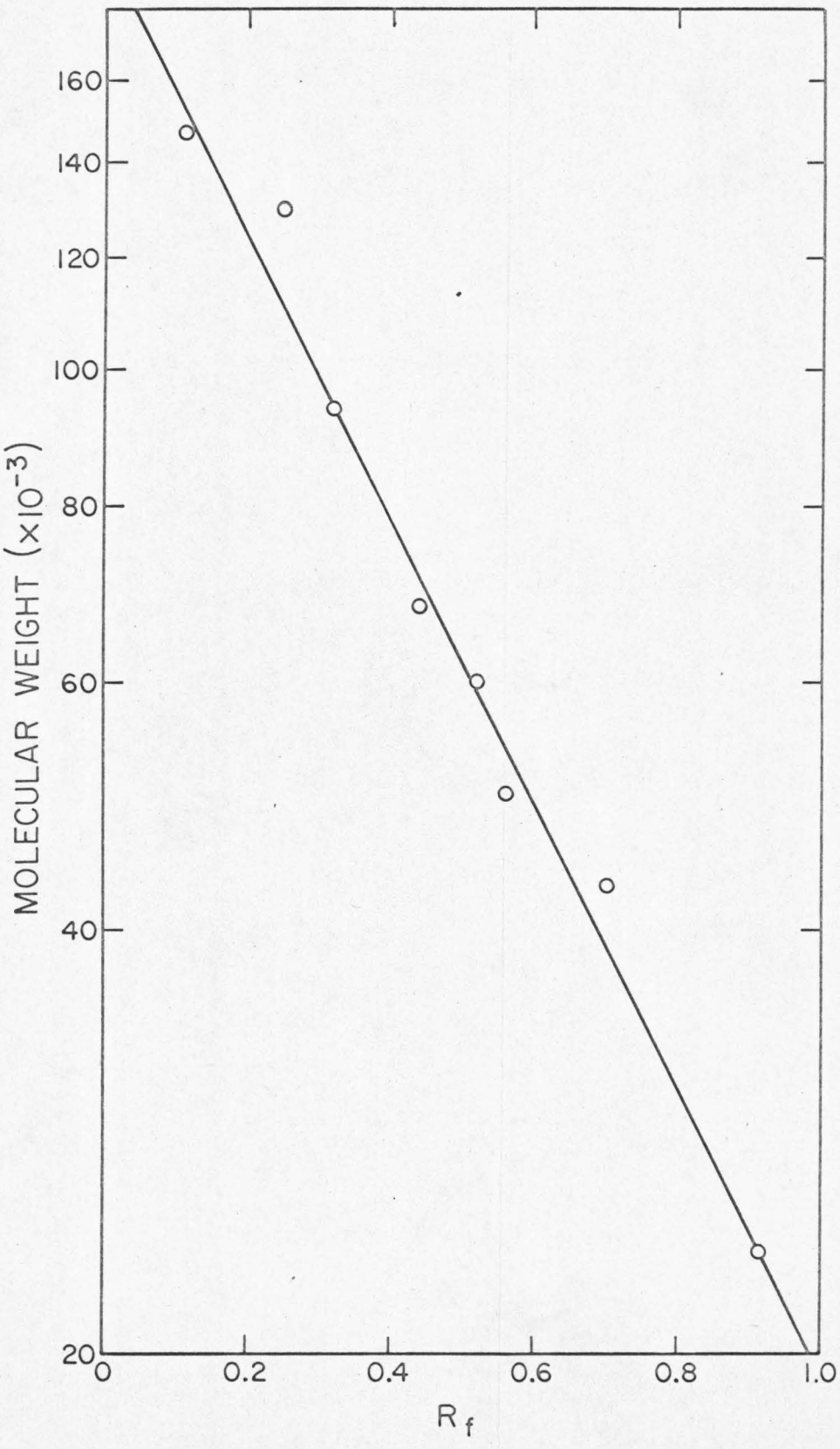

MOLECULAR WEIGHT (x10⁻³)
160
140
120
100
80
60
40
20
0
0.2
0.4
0.6
0.8
1.0
Rf

Fig. 2. Genetic map of T2 gene 34. % recombination is listed below the map and was calculated as ($\underline{am}^+$ recombinants/total progeny) x 200%.

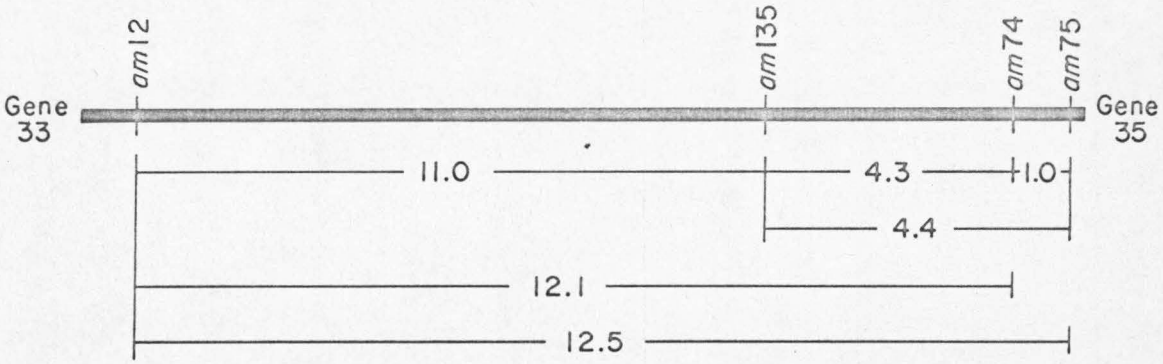
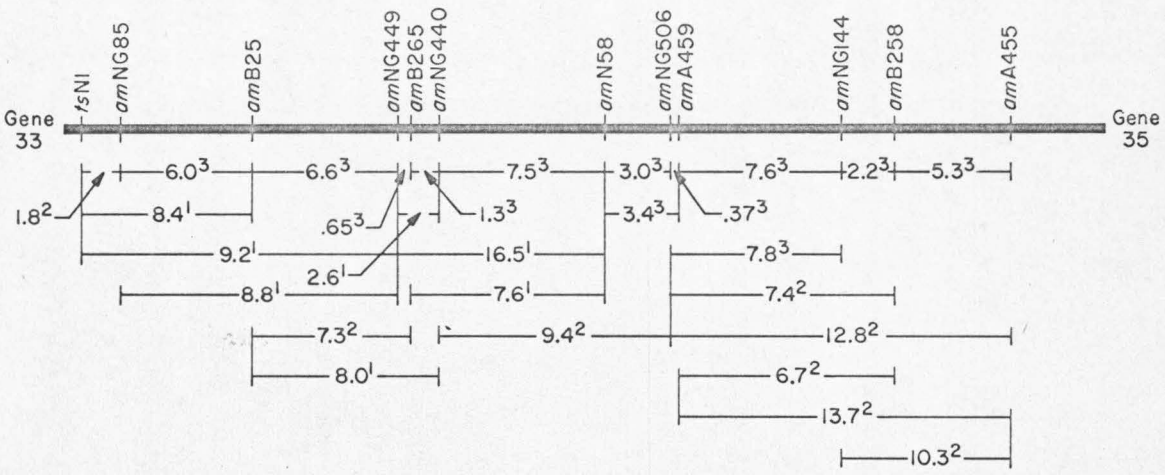

am12
am135
am74
am75
Gene 33
Gene 35
11.0
4.3
1.0
4.4
12.1
12.5

Fig. 3. Genetic map of T4 gene 34. % recombination is listed below the map and was calculated as ($\underline{am}^+$ recombinants/total progeny) x 200%. Superscripts indicate the number of times the recombination in an interval was determined. The order of the non-NG amber mutations agrees with previously published data (Nakata and Stahl, 1967). The ambers designated NG have been ordered as best fits the two factor mapping data. The order of $\underline{am}$NG506 and $\underline{am}$NG144 relative to the mutations which flank them are verified by the physical mapping data.


Gene 33
tsN1
amNG85
amB25
amNG449
amB265
amNG440
amN58
amNG506
amA459
amNG144
amB258
amA455
Gene 35
6.0³
6.6³
7.5³
3.0³
7.6³
2.2³
5.3³
1.8²
8.4¹
.65³
1.3³
3.4³
.37³
9.2¹
16.5¹
7.8³
2.6¹
8.8¹
7.6¹
7.4²
7.3²
9.4²
12.8²
8.0¹
6.7²
13.7²
10.3²

Plates 1 and 2 show band patterns produced by electrophoresis of ^{14}C-labeled lysates of *am* mutants in T2 and T4 genes 34. The position of most of the amber fragments was determined simply by the appearance of a new band. Since the fragment produced during infection with T4 *am*N58 runs very near the third band, P37, the position of the *am*N58 fragment was determined after infection with a double mutant between *am*N52, a mutant in gene 37, and *am*N58. In this way the gene 37 product was removed and the position of the *am*N58 fragment became apparent. The molecular weights of the amber fragments were determined from the standard curve and are listed in Table 1. The physical maps for T2 and T4 were constructed from these data and are presented as the abscissas in Fig. 4.

To compare the genetic and physical maps we calculated, for the interval between two mutations, a ratio of genetic distance to physical distance. This ratio, R_{1000}, is the per cent recombination across the interval (determined from Figs. 2 and 3) per 1000 daltons of protein coded by the interval (determined from Table 1). In Figs. 4a and 4b this ratio, calculated for each appropriate interval in T2 and T4 genes 34, is plotted against the respective physical maps. For T2 gene 34 the value of R_{1000} is relatively low and varies only slightly (.1-.2). In T4 gene 34 over 80% of the gene has a similar low value of R_{1000} (.2). However, in the 20% of gene 34 nearest gene 35 the value of R_{1000} increases sharply reaching 1.7 at the end of the gene.

DISCUSSION

These results demonstrate that the higher frequency of recombination within T4 gene 34 compared with T2 gene 34 is not due to a single

Plate 1. Identification of P34[4] *am* fragments. ^{14}C-labeled lysates were prepared as described in Materials and Methods and electrophoresed on 7.5% polyacrylamide gels containing SDS. After staining and destaining the cylindrical gels were sliced. The slices were dried and autoradiographed on Kodak No Screen X-ray film. For *am*A455, *am*B258 and *am*NG144 the top band is the P34 fragment. The *am*A459 fragment is indicated by the line at the right.

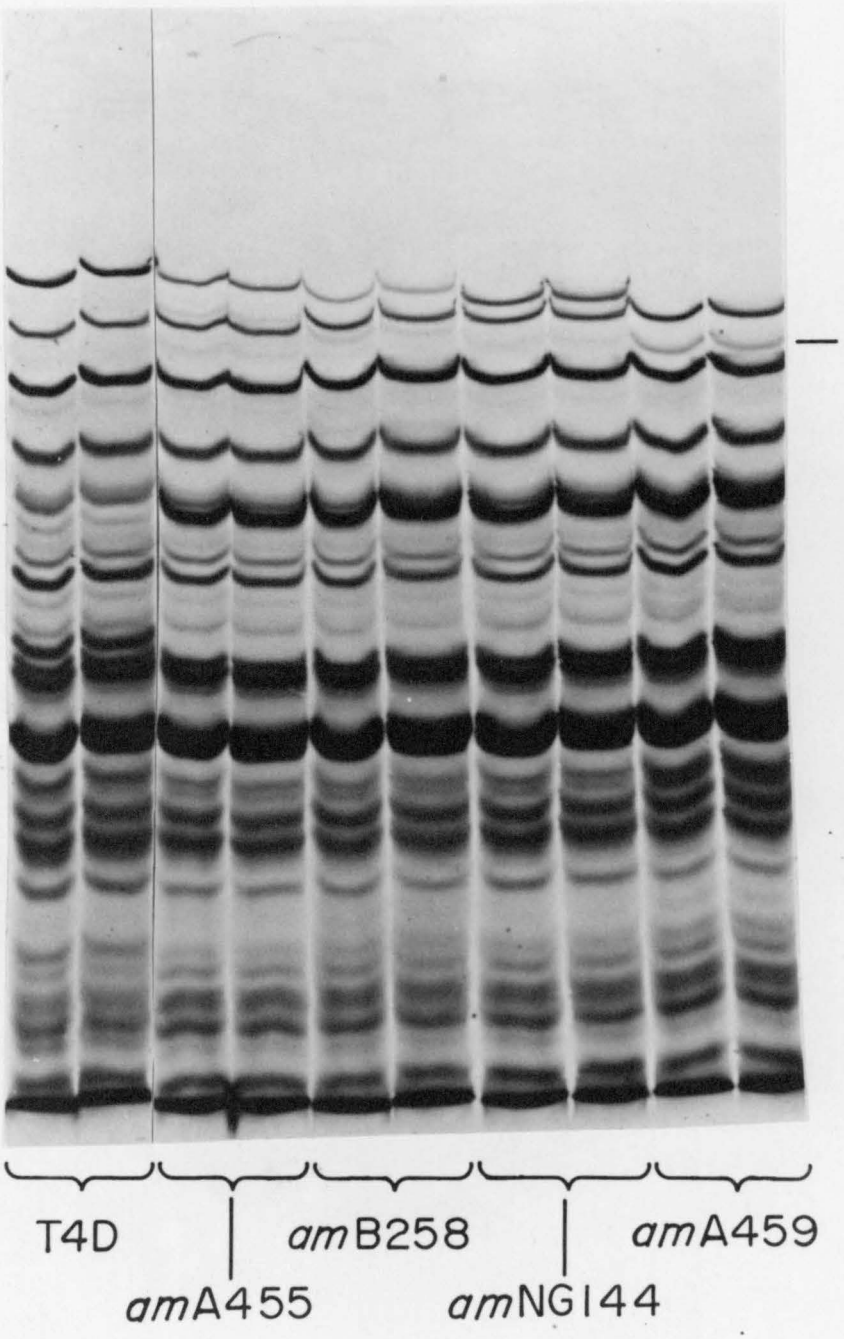

T4D
amA455
amB258
amNG144
amA459

Plate 2. Identification of P34^{2} *am* fragments. For procedure see Plate 1.

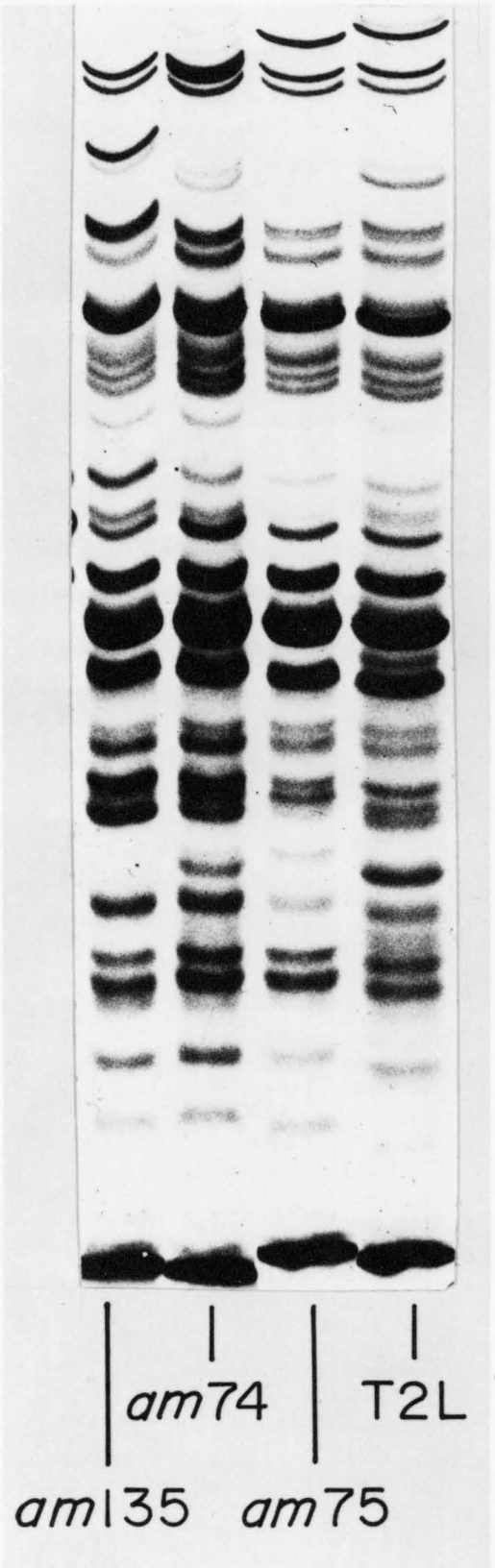

am74
T2L
am135
am75

TABLE 1

Molecular Weights of T2 P34, T4 P34, and Their *am* Fragments

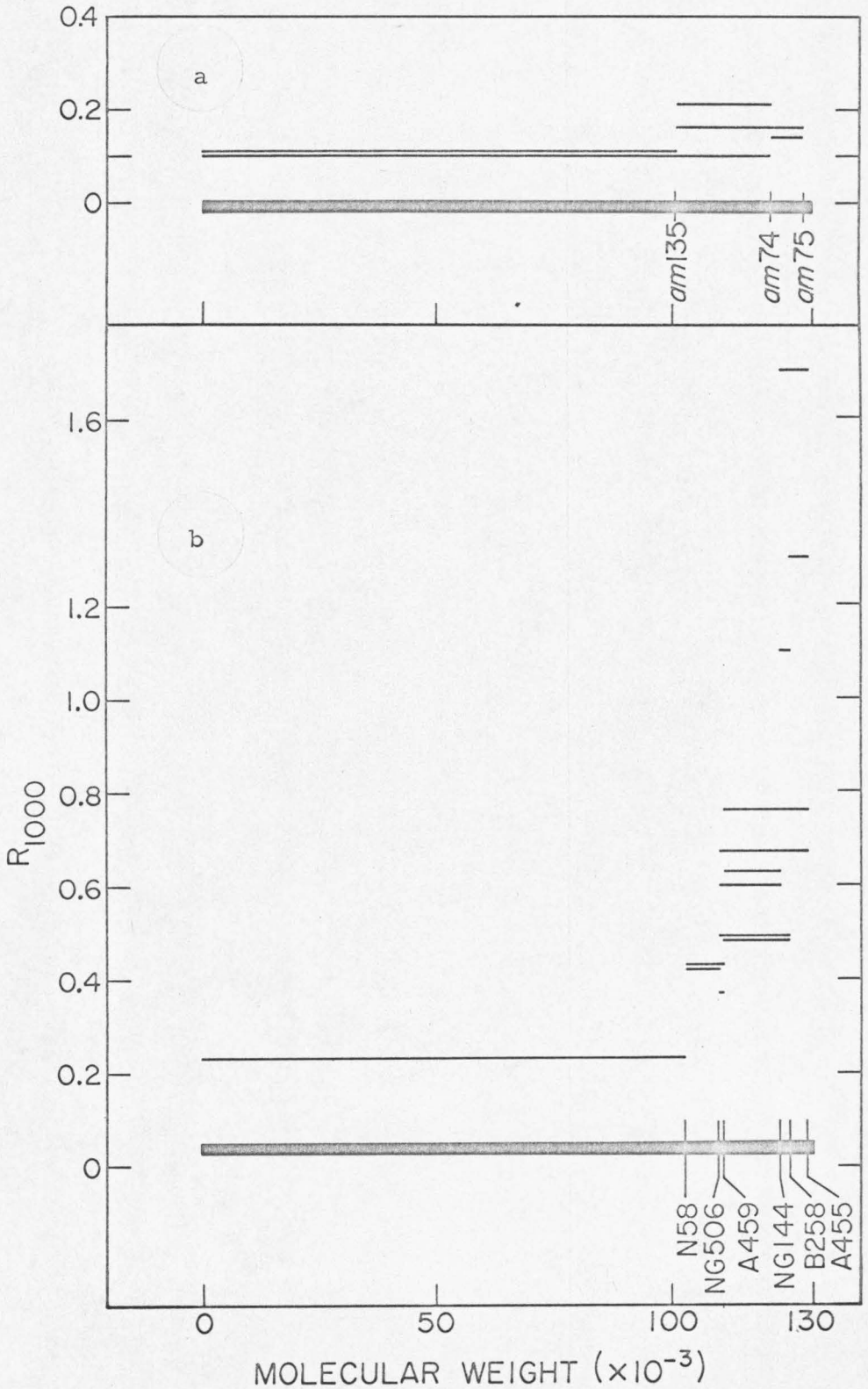
	$MW(X10^{-3})$
T2 P34 *am* Fragments	
*am*l35	101
*am*74	121
*am*75	128
T2 P34	130
T4 P34 *am* Fragments	
*am*N58	103
*am*NG506	110
*am*A459	111
*am*NG144	123
*am*B258	125
*am*A455	129
T4 P34	130

Molecular weights of the gene products and *am* fragments were determined by comparing their migration on SDS polyacrylamide gels with the migration of standard proteins as described in Materials and Methods.

Fig. 4. Comparison between genetic and physical maps of T2 and T4 genes 34. The heavy bar at the bottom of each graph is the physical map of the gene with am mutants located on it. R_{1000}, the percent recombination per 1000 MW of protein is plotted for each interval on the physical map.

a. T2 gene 34

b. T4 gene 34


a
b
0.4
0.2
0
1.6
1.2
1.0
0.8
0.6
0.4
0.2
0
R_{1000}
am135
am74
am75
N58
NG506
A459
NG144
B258
A455
0
50
100
130
MOLECULAR WEIGHT ($\times 10^{-3}$)

recombinational hot spot nor to a homogeneous increase in recombination along the entire length of T4 gene 34. Instead there is a sharp gradient of recombination near one end of the T4 gene, but none in the T2 gene. This gradient extends over at least 700 nucleotides at the end of T4 gene 34 (calculated from Table 1 assuming 3 nucleotides per amino acid and an average residue molecular weight of 110). A sharp increase in R_{1000} near the end of a gene is not characteristic of all T4 genes, however. The value of R_{1000} is nearly constant throughout T4 gene 37 (Beckendorf et al., 1972). Therefore there must be something unique about the structure of gene 34 or the area adjacent to it which causes the abnormally high recombination. However, a unique structure for T4 gene 34, while necessary, is not sufficient to explain the high recombination. T2 gene 34 assumes the same high recombination frequency as T4 gene 34 when it is transferred intact into the T4 genome (G. Rosen and R. L. Russell, personal communication). Thus it appears that some other part of the T4 genome is also necessary to generate high recombination in gene 34. This other function must not exist in T2.

T4 gene 35 also shows increased recombination, so perhaps the gradient of R_{1000} continues into gene 35. However, since P35 has not been identified on gels, we have not been able to extend our studies into gene 35.

Current theories of recombination in T4 and other bacteriophages (Broker and Lehman, 1971; Cassuto et al., 1971) propose that recombination is initiated by single-strand nicks in the DNA. If a fixed site had a high probability of having a single-strand nick, recombination between markers on either side of the nick might be very high. Bacteriophage

T5 may show this effect. There are three nicks in T5 DNA (Abelson and Thomas, 1966; Brejard, 1969) and a recently constructed genetic map of T5 has four very weakly linked groups of markers (Hendrickson and McCorquodale, 1971). One of the nicks seems to separate one of the marker groups, the FST segment, from the rest of the genome (Brejard, 1969). Maximal recombination occurs between markers in this segment and all other markers (Lanni, Lanni and Tevethia, 1966; Hendrickson and McCorquodale, 1971). Although it has not been established whether the other nicks are located between the other groups of markers, it seems reasonable that these nicks are also involved in the high recombination between the groups of markers.

If recombination occurs at a specific site, during T4 infection, there is a high probability of a second recombinational event occurring very close to the first. This probability decreases rapidly with distance away from the first site (Chase and Doerman, 1958). This effect, known as high negative interference, is caused in part by the formation of insertion heterozygotes in which a small single-stranded piece of DNA from one parental phage is inserted into the DNA of the other (Mosig, 1970). When insertion heterozygote DNA is replicated, one of the daughter duplexes is a double recombinant.

These considerations lead to a possible explanation of the recombination gradient in T4 gene 34. The formation of insertion heterozygotes, as well as single recombination events, should be facilitated by the presence of a nick in the DNA. If the site of this nick were fixed, one of the two crossovers necessary for the formation of an insertion heterozygote would occur at this fixed site. The second

would occur adjacent to this site, with decreasing probability at larger distances from the initial site. In other words there would be a decreasing gradient of recombination as the physical distance away from the initial site increases. If this mechanism is responsible for the gradient of R_{1000} we have found in gene 34, there should be, between genes 34 and 35 or in gene 35, a site with a high probability of being nicked. There should also be a gradient, similar to the one we have found but with the opposite slope, on the other side of this nick.

Thus this explanation suggests that the recombination gradient in T4 gene 34 is the direct result of a recombinational hot spot adjacent to gene 34. If this is correct, the abnormally high recombination in gene 34 which we measure is a consequence of secondary exchanges adjacent to the primary exchanges at the hot spot.

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