Analysis of the Structure-Function Relation of Bacteriophage T3 Tail Fiber using T3/T7 Hybrid Phage

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ABSTRACT

The product of gene 17 (gp17) of T3 and T7 phage is the tail fiber protein. Gp17 has multiple functions such as the assembly of monomeric gp 17 to a trimeric tail fiber, the association of tail fibers to the tail, the attachment of phage to its host bacterium and the blocking activity of anti-phage serum (SBP). T3 and T7 are closely related phage but differ in the specificities of these functions of gene 17. We have taken advantage of the different specificities between T3 and T7 to examine functional specialization within the gene.

Hybrid phage with T3/T7 chimeric gene 17 were constructed and their physical maps were determined by *Hpa*I restriction enzyme analysis. Properties of these hybrid phages indicate that SBP is located in a domain in the middle of gp17 while host range determinants are located in a domain in the C-terminus of the polypeptide. SBP is also found in the phage head in the C-terminal part of gp10 (the major head protein). No specificity was detected in the attachment of tail fibers to the tail between T3 and T7. From these results and from comparison of the amino acid sequence deduced from the DNA sequence of gene 17 (T7, Dunn and Studier, 1983; T3, Yamada *et al.*, 1986) the location of functional domain in gp17 and the possible orientation of gp17 in the virion tail fiber are discussed.

INTRODUCTION

Bacteriophage T3 and T7 are closely related. Their genome structures and products are almost identical and many gene products

can be substituted for each ether. However, some gene products exhibit functional specificity and cannot be substituted for each other. The product of gene 1 (gp 1), phage RNA polymerase, cannot transcribe heterogenous DNA efficiently (Dunn *et al.*, 1971) and gp 19, a packaging protein, selects homotypic DNA for packaging into proheads (Fujisawa and Yamagishi, 1981; Yamagishi *et al.*, 1985). Upon absorption of phage to the bacterium, tail fibers interact with specific receptors on the surface of the bacterium. Since *E. coli* BB/7, to which T3 adsorbs but T7 does not, can be isolated, the specificities of adsorption (host range) of T3 and T7 differ. The specificity of adsorption is determined by gp 17 (Issinger *et al.*, 1973).

T3 and T7 differ also in antigenic properties, although some cross reactivity is observed (Adams, 1959). Serum blocking power (SBP), an activity of interference with the neutralizing potency of anti-phage serum, is mainly carried on gp 17 (Issinger *et al.*, 1973; Matsuo-Kato *et al.*, 1981). In addtion to these activities, gp 17 tail fibers are associated with tail proteins on a head at the last step of phage assembly.

Tail fibers of T3 or T7 are trimers of gp 17 (Kato *et al.*, 1985b). They have a total length of 36 nm and are kinked in the middle (Matsuo-Kato *et al.*, 1981). Our interest in the assembly of T3 has led us to examine the assembly process of tail fiber and to characterize the distribution of functional domains within the gp 17 polypeptides. Results by Kato *et al.* (1985a) suggest that C-terminal region of gp 17 plays a role in both initiation of tail fiber assembly and the stability of the mature tail fiber. Although many proteins can be divided into domain structures with different functions, relatively little is known of the relationship between local configuration and functional activities. In this aspect, the T3 tail fiber is an interesting structure because, in contrast to T4 (Wood and Crowthers, 1983), it is composed of only a single gene product, yet has multiple functions, i. e., SBP, the assembly of monomeric gp 17 polypeptides into trimeric tail

fibers, the association to the tail and the attachment of phage to its host. We have taken advantage of the differing specificities between T3 and T7 to examine functional specialization within the gene. Hybrid phages are a useful tool to dissect such structure-function relationship. Analysis of hybrid T3/T7 genes have indicated that the specificity of the transcription of phage RNA polymerase is controlled by a region near its C-terminus (Hausmann and Tomskewics, 1978; Ryan and McConnel, 1982) and that the C-terminal portion of gp 19 is responsible for recognition of specificity in DNA packaging (Yamagishi *et al.*, 1985).

In this paper, we describe the construction of phages with T3/T7 chimeric gene 17. Analysis of the structure and the specificities of the tail fiber of T3/T7 hybrid phage enables us to locate the host range determinant, the antigenic determinant and the orientation of gp 17 in the assembled tail fiber.

MATERIALS AND METHODS

Bacterial and phage strains. *Escherichia coli* R11S and BB were used as permissive and non-permissive hosts, respectively, for amber mutants (Fujisawa *et al.*, 1978). BB/7 was isolated as a derivative of BB on which T3 could adsorb but T7 could not. T3 mutants were from our laboratory stocks and T7 mutants were kindly provided by Dr. Studier (gene number is indicated in parentheses). T3; amNG69 (5), amNG109 (17), amNG169 (17), amNG178 (17): T7; am28 (5), am290 (17). TsNG75 was a temperature sensitive(ts) mutant of T3 gene 17. The genetic map is described in a previous paper (Kato *et al.*, 1985a) and the integration of genetic and physical maps are described elsewhere (Yamada *et al.*, 1986: tsNG75 mutation is located in C-terminal region (*Hpa*I fragment I) and amNG178, amNG109 and amNG169 mutations are located on *Hpa*I fragments M, J and I, respectively (Fig. 1A). T3/T7 hybrid phages, H205 and other H-series phages, and their restriction maps are described in a previous

paper (Yamagichi *et al.*, 1985); the terminal redundancy of H205 is of T3 origin but the most right arm of its genome including gene 17 is derived from T7 genome (Fig. 1B).

Media and buffers. M9 minimal medium supplemented with 0.1% casamino acids (M9A) is described in a previous paper (Fujisawa *et al.*, 1978). EDTA-buffer was 10mM NaCl−10mM Tris-HCl (pH7.4) − 0.25mM EDTA. DF contains lg polypeptone, 3g NaCl and 0.5g $MgSO_4 \cdot 7H_2O$ per liter (Matsuo and Fujisawa, 1975).

Purification of phage DNA. Phages were purified by two cycles of differential centrifugation, followed by banding in CsCl equilibrium density gradient centrifugation and DNA was isolated from purified phage by a standard SDS-phenol method (Fujisawa *et al.*, 1978).

Restriction enzyme analysis. Phage DNAs were cleaved with *Hpa*I and the products were analyzed by electrophoresis on 1% agarose gels or 5% polyacrylamide gels in buffer containing Tris-Borate-EDTA (Yamagishi *et al.*, 1985). Gels were visualized by trans-illumination with uv light and photographs were taken using a Polaroid MP40 camera with Fuji filter SC56 and Polaroid Land coatless film type 667.

Preparation of anti-T3 serum. Anti-serum against T3 phage was obtained from a rabbit by a standard procedure.

Kinetics of neutralization by anti-T3 serum. 0.5m*l* of anti-T3 serum (K=1) dissolved in DF and 0.5m*l* of phage solution (10^8 phages/m*l*) in DF were prewarmed at 37°C for 10 min. Then, they were mixed (time 0) and active phages were titrated at 0, 10 and 20 min after mixing.

Preabsorption of anti-T3 serum. 0.2m*l* portions (K=0.3) were mixed

with 0.4m*l* of tail fibers (10^{10} phage equivalent units (peu)/m*l*), 17^- heads (10^{10} peu/m*l*) or uv-irradiated phage (10^{10} peu/m*l*). The mixtures were incubated at 48°C overnight.

Assay of serum-blocking power (SBP). SBP was measured by a modification of DeMars (1955) as described in a previous paper (Matsuo-Kato *et al.*, 1981).

Extract complementation. Preparation of extracts was according to the methods as described by Matsuo-Kato *et al.* (1981). An extract prepared from cells infected with a particular amber mutant is denoted by the number of the gene having the mutation; 17^- extract is an extract prepared from cells infected with a mutant of gene 17. 5^- and 17^- extracts were mixed and incubated at 30°C for indicated times. Phage were titrated on R11S after appropriate dilutions.

Purification of T3 tail fibers. The procedure of the purification of T3 tail fibers is described in a previous paper (Kato *et al.*, 1985b).

RESULTS

Construction of hybrid phage with T3/T7 chimeric gene 17

To obtain T3/T7 hybrid by recombination between T3 and T7 phage, at least a double crossover is required for hybrid phage to be viable, because the terminally redundant sequences of T3 and T7 are different from each other (Fujisawa and Sugimoto, 1983) and because the terminal sequences are essential for phage DNA replication (Watson, 1972). Therefore, the frequency of hybrid phages is very low. However, when a T3/T7 hybrid phage, which has T7 gene of interest and T3 terminal redundancy, is crossed with T3 phage, a single cross-over in the gene would make viable hybrid(s).

To construct T3/T7 hybrid in gene 17, double mutants of am(s) and ts mutants of T3 were initially constructed by usual genetic

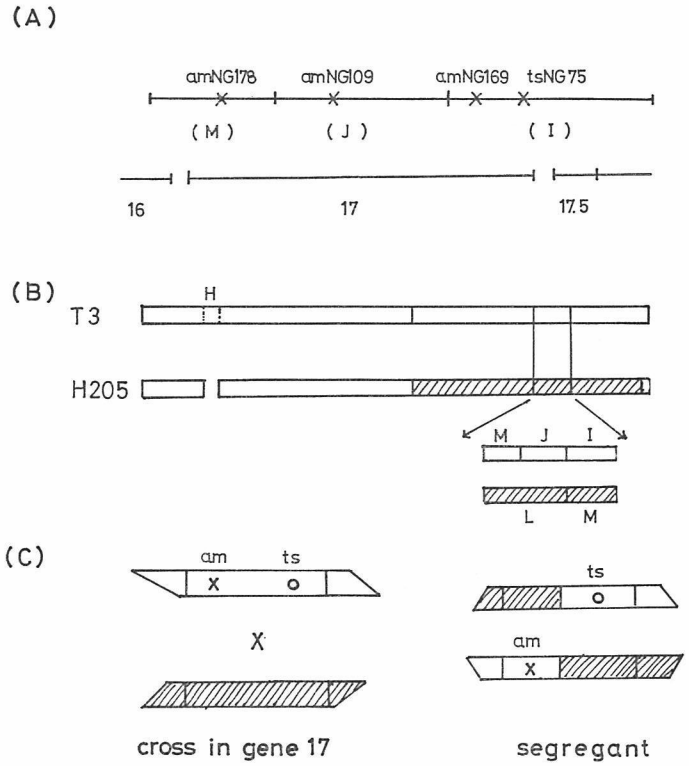

Fig. 1 Construction of T3/T7 hybrid phages with chimeric gene 17.

(A) Genetic and physical maps of T3 gene 17 (Yamada *et al.*, 1986). Mutation sites have not determined at DNA sequence level.

(B) Physical maps of T3 and T3/T7 hybrid H205. *Hpa* I fragments of interest are shown. The open and shaded boxes represent T3 and T7 sequences, respectively (Yamagishi *et al.*, 1985).

(C) Diagrammatic structure of gene 17 of am or ts segregant obtained by crossing T3 am-ts recombinants and H205 phage.

crosses. Then, these am-ts recombinants were crossed with H205, a T3/T7 hybrid phage, which has T7 gene 17 and T3 terminal redundancy (Yamagishi *et al.*, 1985, Fig. 1B) and am or ts segregants were selected. Gene 17 of an am-segregant is predicted to be

composed of T3 N-terminal and T7 C-terminal portions and that of a ts-segregant is predicted to be composed of T7 N-terminal and T3 C-terminal portions (Fig. 1 C). With the use of amber mutations lying closer to the ts mutation site, crossover sites nearer to the C-terminus of gene 17 will be selected (Fig. 4). 2−5% of the total progeny phage from such crosses were isolated as am-or ts-segregants (Table 1) and representatives were characterized as follows.

Table 1
Construction of hybrid phage with T3/T7 chimeric gene 17

T3 recombinant crossed with H205	Hybrid phage	
	am segregants	ts segregants
amNG178-tsNG75	am1A, am15A	ts1A, ts2A
amNG109-tsNG75	am1B, am2B	ts1B, ts2B
amNG169-tsNG75	am3C, am5C	ts1C, ts2C

Restriction enzyme analysis of T3/T7 hybrid with chimeric gene 17

To confirm the above prediction on the structure of T3/T7 chimeric gene 17, T3/T7 hybrid phage DNAs were analyzed by *Hpa*I. Gene 17 of T3 is distributed on three *Hpa*I fragments of sizes of 507 bp(M), 873 bp(J) and 1005 bp(I) (Yamada *et al.*) while gene 17 of T7 is distributed on two fragments of sizes of 1380 bp(L) and 996 bp(M) (Dunn and Studier, 1983) (Fig. 1A). DNA sequence analysis has shown that T3 *Hpa*I site between fragments J and I is conserved as T7 *Hpa*I site between fragments L and M (data not shown). Marker rescue tests indicated that amNG178, amNG109 and amNG169 mutations were located on fragments M, J and I, respectively and that tsNG75 was located on fragment I (Yamada *et al.*, 1986).

Hybrid phage DNAs were digested with *Hpa*I and subjected to electrophoresis on agarose or polyacrylamide gel as described in Materials and Methods (Fig. 2). *Hpa*I fragments in hybrid phage DNAs were identified as T3 or H205 counterparts on the bases of size. Specifically, the presence or absence of the following fragments

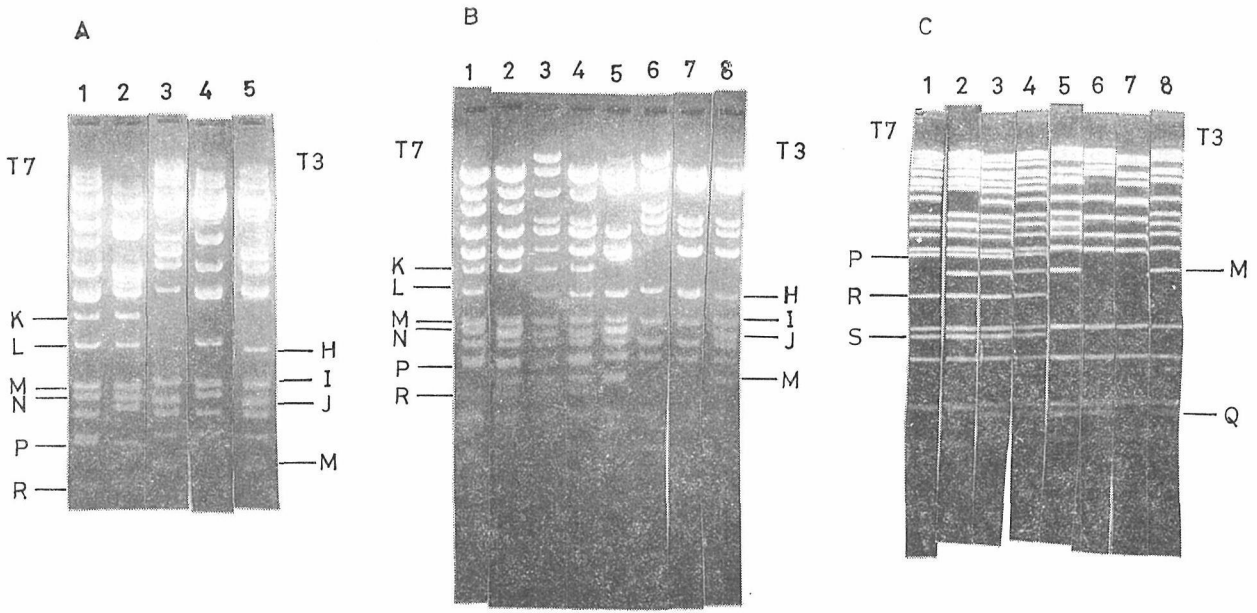

Fig. 2 *Hpa*I digests of T3, H205 and T3/T7 hybrid phage DNAs.
(A) 1.6% agarose, (B) 1.5% agarose, (C) 5% polyacrylamide.
The letters identify the T3 and T7 *Hpa*I fragments. (A) 1; H205, 2; T7, 3; am1A, 4; ts1A, 5; T3. (B) and (C) 1; H205, 2; am15A, 3; am1B, 4; am2B, 5; am3C, 6; ts2B, 7; ts1C, 8; T3.

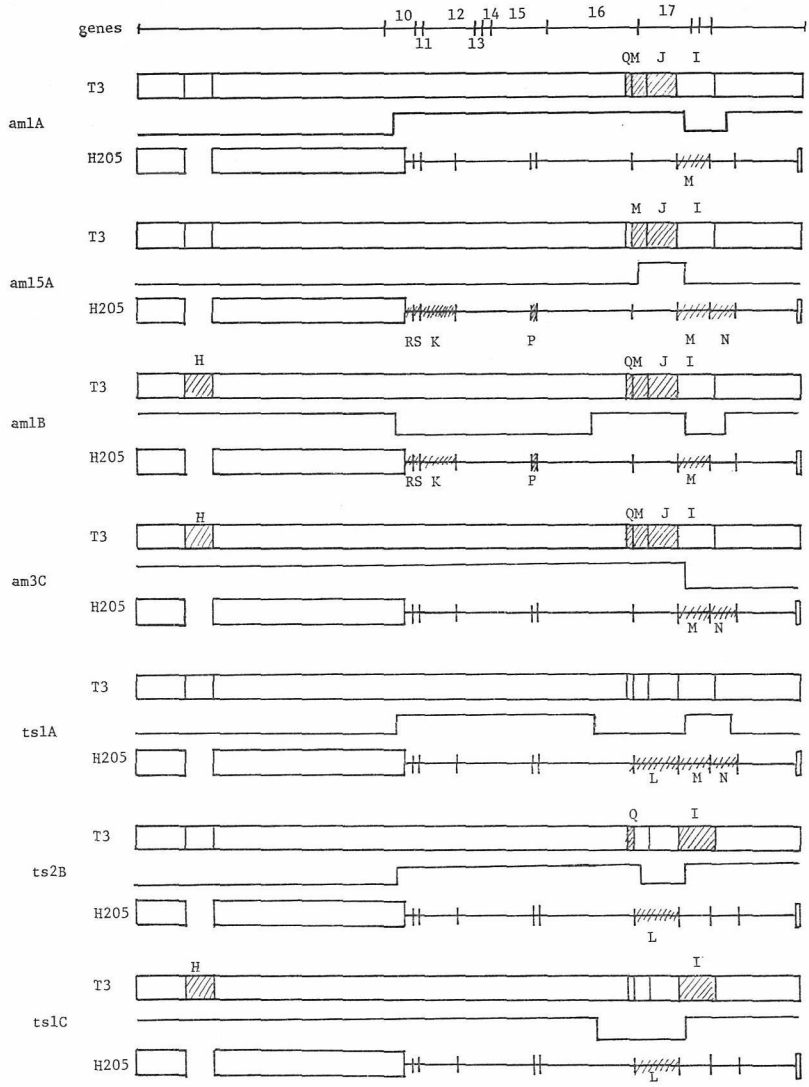

Fig. 3 Diagrammatic representation of the most likely patterns of crossovers between T3 recombinants and H205.

Hpa I sites are indicated. Fragments identified in Fig. 2 are shaded. Restriction maps of H-series are described in a previous paper (Yamagishi *et al.*, 1985).

were checked: T3 H, Q, M, J, I and E; T7 3420bp, R, S, K, P, L, M and N (Yamagishi *et al.*, 1985). It was difficult to distinguish T3 fragment I (1005 bp) and T7 fragment M (995 bp). Probable cross-over sites were deduced from restriction patterns (Fig. 3). Although it was difficult to determine from restriction enzyme analysis whether a cross-over occured between T3 fragment I and T7 fragment M or between T3 fragment J and the right half of T7 fragment L, a cross-over must have occured between 1128th and 1377th nucleotide from the A of the intiation ATG codon of gene 17 (*Hpa*I site between T3 fragments J and I, 1284-1289th nucleotide) where DNA sequences are highly conserved between T3 and T7 (Yamada *et al*, 1986). In the case of am1A and am2B, the crossover back to T3 sequence must have occured within T7 fragment M or N because of the absence of tsNG75 mutation and T7 fragment N. Similarly, in the case of ts1A, the crossover back to T7 sequence must have occured within T7 fragment M or N because of the presence of tsNG75 mutation and T7 fragment N. As shown in Fig. 3, additional crossovers seem to occur at a few fixed sites as described in a previous paper (Yamagishi *et al.*, 1985).

Host range determinant

To locate the host range determinant, we have tested whether hybrid phages have T3 or T7 host range (h3 or h7, respectively) by plating on BB/7. As shown in Table 2, all ts-segregants were h3 and, in contrast, all am segregants were h7. Since the tsNG75 mutation is located in the C-terminal portion of gene 17, these results indicate that the host range determinant is located in C-terminal region of gp 17. The same conclusion was obtained from the host range analysis of H-series hybrid phages isolated in a previous paper (Table 3, Yamagishi *et al.*, 1985); a hybrid phage was either h3 or h7 according to the presence of C-terminal region of T3 or T7, respectively. The host range appears to be not controlled by gp 10, gp

Table 2
Host Range of T3/T7 Hybrid Phages

Hybrid phage	Host range
am1A am15A	h7
am1B am2B	h7
am3C	h7
ts1A	h3
ts1B ts2B	h3
ts1C ts2C	h3
H101	h7
H104	h7
H001	h3
H305	h3
H501	h7
H205	h7

Host range of hybrid phage was determined by measuring its plating efficiency on *E. coli* BB/7 relative to *E. coli* BB. The plating efficiency of am series was determined on am^+ revertant.

11 or gp 12 as judged from restriction maps of hybrid phages (Fig. 3).

Neutralization of T3/T7 hybrid phage by anti-T3 serum

Since the main SBP of T3 phage is carried on gp 17 as shown in a previous paper (Matsuo-Kato *et al.*, 1981) and the antigenic specificities are different between T3 and T7, it is possible to determine the distribution of antigenic determinants on gp 17 by examing the antigenic properties of hybrid phages. Kinetics of neutralization of hybrid phages by anti-T3 serum were examined by determining the survivors after mixing phage and anti-T3 serum as described in Materials and Methods. As shown in Fig. 4, neutralization kinetics of T3 and T7 were different from each other, with T7 reacting less rapidly with anti-T3 serum. Hybrid phages with chimeric gene 17

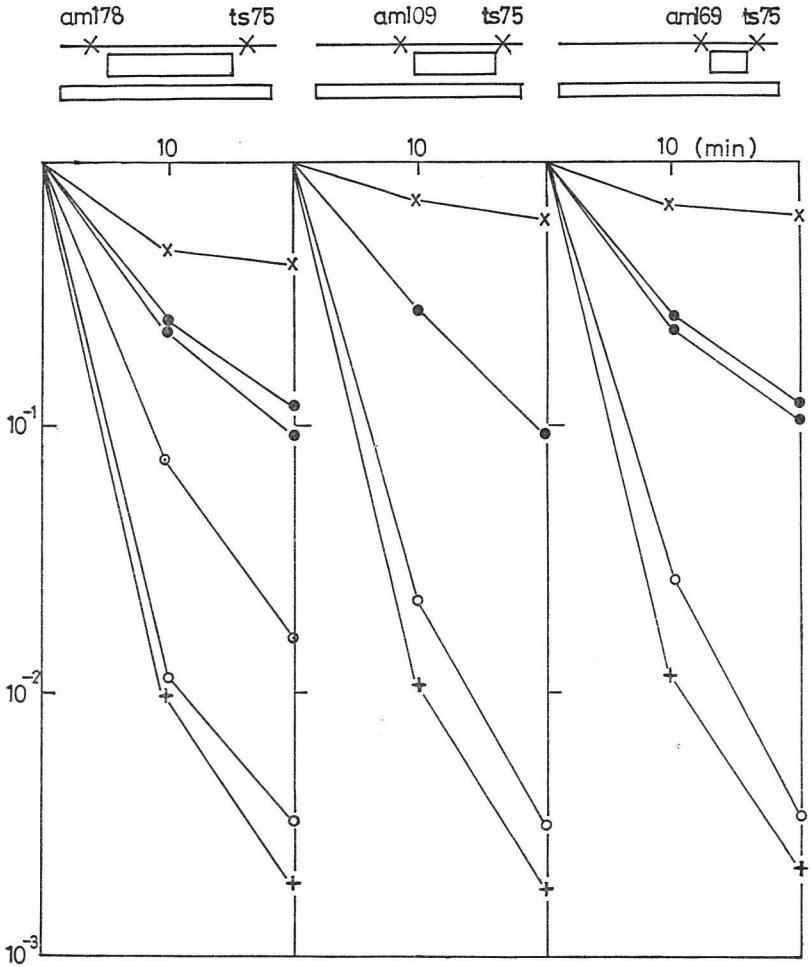

Fig. 4 Neutralization kinetics of hybrid phage by anti-T3 serum.
The upper section shows the region of the possible crossover in gene 17. The lower section indicates inactivation of phages by anti-T3 serum. Reactions were performed as described in Materials and Methods. —+— T3, —×— H205, (1) —●— ts1A and ts2A, —⊙— am15A, —○— am1A. (2) —●— ts2B, —○— am2B. (3) —●— ts1C and ts2C, —○— am3C.

appear to be divided into three groups depending on their kinetics of neutralization. Group 1 phage (am1A and am3C) was indistinguishable from T3 phage. Group 3 phage (ts1C and ts2B) was moderately neutralized by anti-T3 serum and group 2 phage (am15B) showed kinetics intermediate between the groups 1 and 3 (Fig. 4). The same kinetic experiments were performed with H-series hybrid phages.

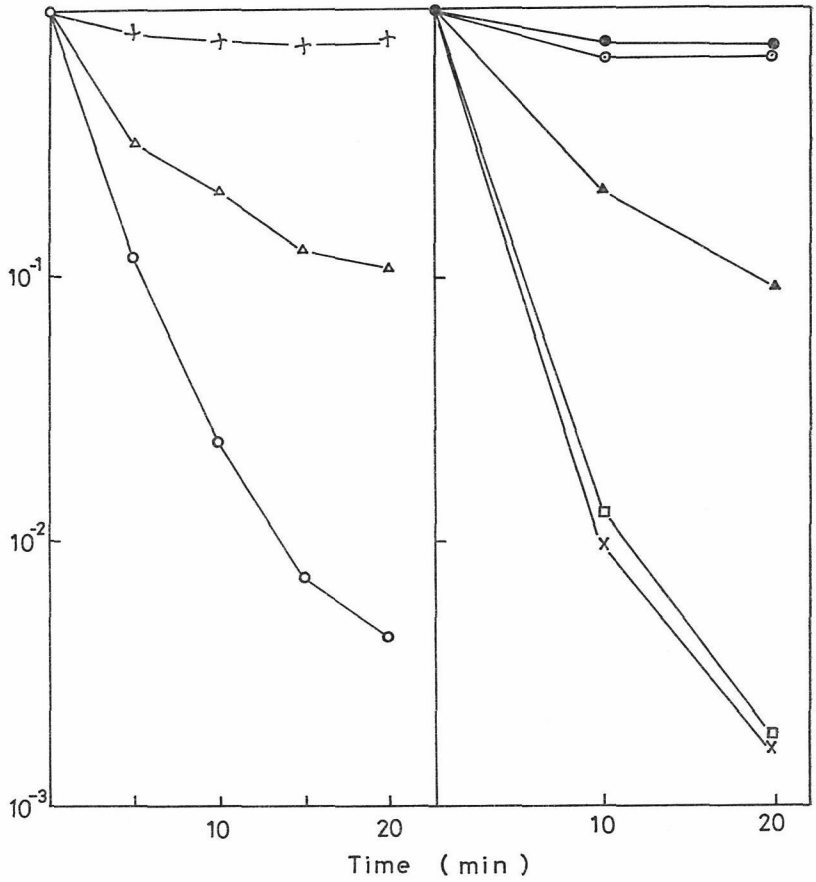

Fig. 5 Neutralization of H-series hybrid phage by anti-T3 serum.
Inactivation experiments were performed on H-series T3/T7 hybrid phages as described in the legend to Fig. 4. (1) —+— T7, —△— H001, —○— H104, (2) —●— H205, —◎— H501, —▲— H305, —□— T3. —×— H101.

These hybrid phages were divided into three groups, two groups similar to those above groups (group 1, H101; group 2, H104; group 3, H305 and H001) and group 4 phage (H501) showing the kinetics indistinguishable from T7 (Fig 5).

Serum blocking power (SBP)

To explain the different groups of hybrid phages with reduced rates of serum inactivation relative to T3, it is possible that T3 phage antigenicity is composed of a number of different antigens and that hybrid phages would receive only a fraction of these antigens from T3. Thus, hybrid phages would interact with only a few classes of

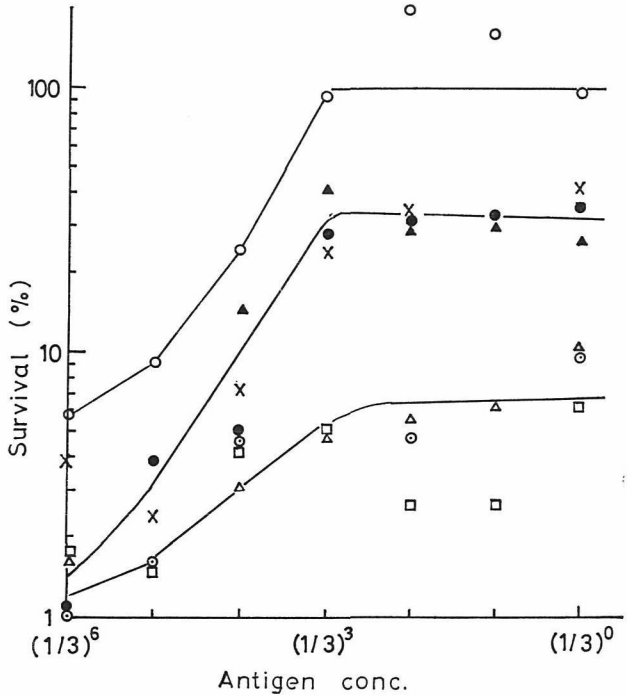

Fig. 6 Blocking of anti-T3 serum by various antigens.
Serum blocking was carried out as described in Materials and Methods. —○— T3 wild phage; —△— H305; —●— am15B, —×— H104; —▲— T3 tail fibers; —□— 17$^-$ particles; —◎— 12$^-$ particles.

antibodies in the serum and be killed at a lower rate than T3. To examine the possibility, the serum was incubated with an increasing amounts of tail fibers or 17⁻ particles until the reaction has gone to completion and tested for the amount of serum activity which has been eliminated or blocked.

As shown in Fig. 6, the decrease in serum activity continued with increasing amounts of antigens and leveled off at certain amounts of the antigens. The percentage by which the original K-value is decreased when antigen is excess, is a measure of the percent of the antibodies which was able to combine with the antigens of the sample. The K-values were determined from the data on the rate of neutralization by anti-T3 serum preabsorbed by an excess amounts of the antigen (Table 3). The result shows that SBP of T3 is composed of one (74.6%) derived from tail fibers and other (24.9%) derived from tail fiberless particles (17⁻ particles).

Restriction maps (Fig. 3 Yamagishi *et al.*, 1985) predict that H104 and H305 virions are composed of T7 head-T3 tail fibers and T3

Table 3
Neutralization of hybrid phages by anti-T3 serum preabsorbed against phage antigens

		Anti-T3 serum preabsorbed against	
Phage	none	T3 tail fiber (As−T)	T3 17⁻ particle (As−H)
T3 wild	1.7	36.1	11.8
am3C	1.9	30.8	6.1
H101	1.2	34.5	8.3
am15A	1.5	100	0.8
H104	1.3	100	0.8
am1B	18.0	100	12.1
ts2B	25.6	32.0	100
H305	49.8	41.2	100
H001	45.6	37.2	100

Neutralization experiments were performed as described in Materials and Methods. Survivors(%) after 60 min incubation at 37C are indicated.

head-T7 tail fibers, respectively. H104 and H305 behaved in the same manner in serum blocking as tail fibers and 17⁻ particles, respectively (Fig. 6). H104 and H305 were treated with the serum preabsorbed against tail fiber (AS−T) or 17⁻ particles (AS−H). As shown in Table 3, H305 was neutralized by AS−T but not at all by AS−H and on the contrary, H104 was neutralized by AS−H but not at all by AS−T. The results seems to be consistent with the predicted protein compositions of hybrid phage virions. In the following experiments, anti-T3 serum preabsorbed against H104 or H305 was used as equivalents to AS−T or AS−H, respectively. As shown in Table 3, group 3 phage (ts2B, H305 and H001) was neutralized by AS−T as well as by anti-T3-serum (AS−0) but not at all by AS−H. On the contrary, Group 2 phage (am1B) was neutralized by AS−H, as well as AS−0 but not at all by AS−T. The results indicate that phage belonging to group 2 and group 3 are composed of T7 head-T3 tail fibers and T3 head-T7 tail fibers, respectively. To confirm the conclusion, chimeric phage composed of T3 head and T7 tail fibers or of T7 head and T3 tail fibers, were constructed by extract complementation, purified by CsCl banding centrifugation as described in a previous paper (Matsuo and Fujisawa, 1975) and treated with anti-T3 serum. As shown in Fig. 7, chimeric phage composed of T7 head-T3 tail fibers or T3 head-T7 tail fibers were nuetralized in kinetics similar to those of group 2 or 3, respectively. Although group 1 phage were neutralized by anti-T3 serum in the same kinetics as T3 phage, they were divided into two subgroups, A and B, depending upon their antigenicities. Am3C and H101 (group 1A) were neutralized to the same extent as T3 by AS−T and AS−H. The results indicate that these phages in group 1 are composed of T3 head-T3 tail fibers. However, H104 and am15A (group 1B) were neutralized at a higher rate by AS−H than T3 phage but not at all by AS−T (Table 3). The results indicate that these hybrid phage are composed of T7 head and T3 tail fibers. To explain the increased rate of neutralization

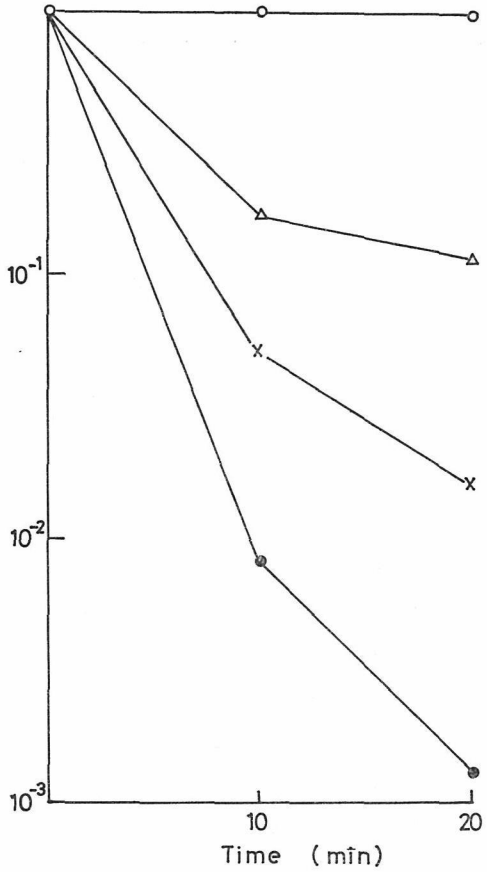

Fig. 7 Neutralization of chimeric phage constructed by *in vitro* complementation.

Chimeric phage were constructed by *in vitro* complementation and purified by CsCl step gradient centrifugation as described by Matsuo-Kato *et al.* (1981). Inactivation of phages was performed by anti-T3 serum preabsorbed against T7 as described in the legend to Fig. 4. —○— T7 head + T7 tail fiber, —△— T3 head+T7 tail fiber, —×— T7 head+T3 tail fiber, —●— T3 head +T3 tail fiber.

by AS—H, it is probable that antigenic site(s) buried in T3 tail fiber is exposed on tail fiber composed of T3/T7 chimeric polypeptides. T3 phage was inactivated to the same extent by anti-T3 serum

preabsorbed against H104 virion as by AS−T (data not shown). The fact shows that a new class of antigenic site is not exposed on the tail fiber of H104.

The specificity of the assembly of tail fibers to tailed heads

The final step of T3 phage assembly is association of tail fibers to 17⁻ particles. To determine the specificity in the association of tail fibers to 17⁻ particles, *in vitro* cross-complementation experiments were performed between T3 and T7.

As shown in Fig. 8, the kinetics of the association of tail fibers to 17⁻ particles were indistinguishable between homotypic and hetrotypic combinations.

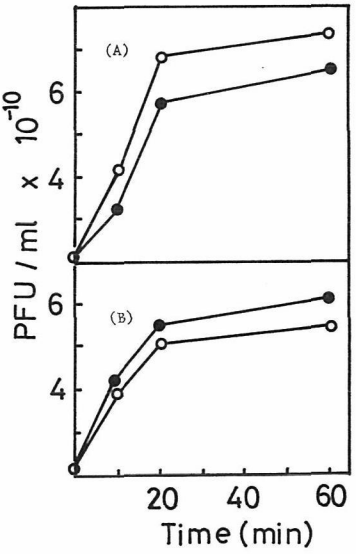

Fig. 8. Kinetics of phage production by *in vitro* complementations between 17⁻ and 5⁻ extracts of T3 and T7.

Extract complementations were performed between T3 (A) or T7 (B) 17⁻ extract and T3 and T7 5⁻ extracts as described in Materials and Methods. (A) —○— T3 5⁻ 10⁻ extract, —●— T7 5⁻ 10⁻ extract. (B) —●— T3 5⁻ 10⁻ extract, —○— T7 5⁻ 10⁻ extract.

DISCUSSION

The serum bloking experiments showed that there are at least two antigens, head-and tail fiber-related antigens, in T3 virions. Hybrid phages can be divided into five groups depending on the combinations of these two antigens; group 1A, T3 head-T3 tail fibers; group 1B, T7 head-altered T3 tail fibers; group 2, T7 head-T3 tail fibers; group 3, T3 head-T7 tail fibers and group 4, T7 head-T7 tail fibers. This classification is consistent with the protein compositions predicted by restriction maps of hybrid phages. H205 had the same antigenicity as T7 and H305 had T3 head-related antigenicity (Table 3). The restriction maps indicate that there is no difference in virion components between H205 and H305 except that the major head protein (gp 10) of H205 is a hybrid protein composed of T3-N terminal and T7-C terminal portions but gp 10 of H305 is of T3 origin. The results suggest that head related antigenic determinants are located in the C-terminal portion of gp 10 and not in other head components (gp 8, 13, 14, 15 and 16). Although H001, ts1A, ts2B and ts1C virion were composed of T3 head, T3 tail proteins (gp 11, 12) and T7 tail fibers, it had only T3-head related antigen. The fact indicates that tail proteins, gp 11 and gp 12, do not carry serum blocking activity. The conclusion is supported by the fact that SBP was indistinguishable between 17^- and 12^- particles (Fig. 6).

The ts segregants ts1A, ts2B and ts1C had no T3 tail fiber related antigen. H501 had T3 fragment M but no T3 tail fiber antigen. Therefore, antigenic determinant(s) of T3 tail fiber appears to be located in T3 fragment J. The kinetics of neutralization by AS−H of hybrid phage which belonged to groups 1A and 2 was identical among hybrid phages and indistinguishable from that of T3 phage. The results imply that tail fibers of these hybrid phage carry antigen(s) common to T3 tail fibers. Group 1A phage were neutralized to the same extent as T3 phage by anti-T3 serum but at a higher rate by

AS−H than T3 phage. The increased antigenicity appears to be caused by appearence of the same antigen as T3 tail fibers probably due to altered conformation of T3/T7 chimeric tail fibers. It remains to be determined whether the common antigen is composed of a single antigen or multiple subclasses of antigens. In the latter case, if recombination occurs more frequently at the exterior of multiple antigenic determinants than in the middle, they would behave as a single antigen. From the analysis of T2/T4 hybrid phage, Beckendorf (1973) suggests that T4 gp 37 in the distal half-fiber is composed of a number of sub-sets with different specificities, located in distinct regions of the half-fiber.

The analysis of the host range of hybrid phages indicate that host range determinants are located in the C-terminal portion of gp 17. The host range of several phage is reported to be determined by the C-terminal part of the tail fiber protein (T2-T4; Beckendorf *et al.*, 1973; lambdoid phages, Simons *et al.*, 1971).

In summary, it is concluded that host range and antigenic determinants in gp 17 are located in different domains. The host range determinants are in the C-terminal region (coded by DNA sequence on T3 *Hpa*I fragment I) and the antigenic determinants are in the middle of the gene (coded by DNA sequence on T3 *Hpa*I fragment J). As described elsewhere (Yamada *et al.*, 1986), the nucleotide sequence of gene 17 has been determined. The coding sequence directs the synthesis of a polypeptide of 557 amino acids, although T7 gene 17 encodes a protein of 554 amino acids (Dunn and Studier, 1983). Comparison of the amino acid sequences deduced from DNA sequences of T3 and T7 genes 17 shows that there are four domains in gp 17 as judged by analysis of amino acid homology. The N-terminal domain is fairly conserved (residues 1-202, 97% homology). The middle domain is less conserved (residues 203-375, 76% homology) and separated from non-homologous C-terminal domain with different length between T3 and T7 (T3, residues 460-557; T7, residues 460-554)

by a region with relatively high homology (residues 376-554, 91.5% homology). The middle and C-terminal domains of gp 17 would include antigenic and host range determinants, respectively.

Phage adsorbs to the bacterial surface by the distal part of tail fibers. The distal part should correspond to the C-terminal domain of gp 17 because the domain determines the host range of T3 and T7. In turn, the proximal part which is associated with tail appears to correspond to N-terminal domain. The assumption is consistent with the facts that N-terminal protein sequence is highly conserved and that gp 17 of T3 and T7 are exchangeable during *in vitro* assembly of tail fibers to the tail. From these considerations, we conclude that gp 17 molecules in the tail fiber are oriented parallel each other in order to associate to the tail with its N-terminal domain and to attach to the host bacterium through its C-terminal domain. The high homology of the N-terminal domain suggests that the functional specificity of N-terminal domain has apparently not changed during the divergence of T3 and T7. Interestingly, these features of conservations of sequences are commomly observed in the tail fiber genes (T2-T4, lambdoid phages). These common trends during divergence of related phages suggest that the sequence of the proximal part which contacts other phage proteins is structurally constrainted and that the distal part which interacts with the bacterial surface undergoes sequence changes which are favorable for phage to extend host range properties.

ACKNOWLEDGMENTS

I am grateful to Dr. F. Fujimura, La Joola Cancer Foundation, for invaluable help with the manuscript, and Dr. Ryo for his discussion.

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T3とT7の雑種ファージを用いた T3尾部繊維の構造と機能の解析

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要約

T3ファージやT7ファージの遺伝子17産物(gp 17)は尾部繊維の構造タンパク質である。gp 17は多くの機能をもっている。例えばgp 17が3分子集合して一本の尾部繊維を作る，尾部繊維は尾部に結合する，ファージが宿主である細菌に吸着するための器官であり，抗血清阻止力(serum blocking power, SBP)をもっている。T3とT7は密接に関連したファージであるがgp 17のこれらの機能には各々のファージで特異性がある。それゆえに遺伝子17についてT3とT7の間で機能的な特異性部位を決定する研究を行うことができる。

遺伝子17の中でT3とT7が雑種になっているファージを作った。そしてそれらのファージのDNAをHpaI制限酵素で切った消化DNAパターンにより制限酵素地図を作製した。これらの雑種ファージの性質はSBPがgp 17の中央付近に位置し，宿主域はgp 17のC端にあることを示唆した。SBPはファージの頭部主要タンパク質(gp 10)のC端にも見つかった。

尾部繊維が尾部に結合することに関してはT3とT7の間で特異性はなく互換的であった。これらの結果とT3とT7の遺伝子のDNA塩基配列から帰結されたアミノ酸配列を比較してgp 17の機能部位を考えた。

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