SEROLOGICAL STUDIES WITH MUTANTS OF PHAGE T4D DEFECTIVE IN GENES DETERMINING TAIL FIBER STRUCTURE¹

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RECENT studies of conditional lethal mutations of phage T4D have revealed the existence of a large number of genes which control the morphogenesis of the virus particle (EPSTEIN, BOLLE, STEINBERG, KELLENBERGER, BOY DE LA TOUR, CHEVALLEY, EDGAR, SUSMAN, DENHARDT and LIELAUSIS 1963). While these genes appear to play relatively discrete roles in the morphogenetic process, affecting only the construction of particular virus components, multigenic control of component synthesis and assembly is general. Studies by SARABHAI, STRETTON, BRENNER and BOLLE (1964), for example, showed that gene 23 controls the synthesis of the major protein component of the virus head membrane, yet at least five other genes are necessary in active form for the construction of normal head membranes. Knowledge of the roles these many genes play in the construction of specific virus components is crucial to an understanding of their structure and mode of assembly.

For most of the morphogenetic genes, the component of the virus particle affected by mutation is not yet known. However, it has been shown that genes 34, 35, 36, 37, 38 and 57 are all concerned with the construction of the tail fibers of the phage (EPSTEIN *et al.* 1963; EDGAR and EPSTEIN, unpublished). Mutations that affect host range (STREISINGER 1956) and co-factor requirement (BRENNER 1957) are located in the 34–38 region of the genome, as are the genetic determinants of antibody neutralization specificity (STREISINGER 1956). It would appear that the tail fiber gene cluster offers favorable material for investigations into the nature of multigenic control of virus components.

The work reported here concerns the antigenic properties of conditional lethal mutants defective in this region of the genome. It will be shown that at least three distinct antigens are involved in the neutralization of phage by antibody and that two of these antigens are under multigenic control. A new gene, gene 57, unlinked to the 34–38 cluster, was found to specifically affect the production of all three antigens. A regulatory role for this gene is possible.

MATERIALS AND METHODS

Phage strains: Various amber (*am*) and temperature sensitive (*ts*) mutants of phage T4D defective in tail fiber genes were used and have been described elsewhere (EPSTEIN, *et al.* 1963; EDGAR and LIELAUSIS 1964; EDGAR, DENHARDT and EPSTEIN 1964). Maps of the tail fiber region

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FIGURE 1.—A map of T4D showing the relative locations of the tail fiber genes and a few other genes for reference.

FIGURE 2.—A map of the gene 34-38 region of the genome. The relative locations of mutations reported in the text are shown. The mutants *amC290*, located in gene 38, and *tsCB110*, located in gene 36, have not been precisely mapped.

of the genome indicating the relative locations of the mutations pertinent to this study are shown in Figures 1 and 2. The *rII* mutants, *ra61*, a nonreverting mutant in the *A* cistron, *rd41*, a deletion encompassing the *A* and *B* cistrons, and the *rI* mutant, *r48*, were also used.

Bacterial strains: *Escherichia coli* strain CR63 was used as permissive host for amber mutants, strain B and its derivative S/6 as restrictive hosts. Strain F(λ) (STEINBERG and EDGAR 1962) was used as plating indicator for the tester phage in the serum blocking experiments as it is restrictive for both amber and *rII* mutants. Restriction of temperature sensitive mutants was accomplished by high temperature (39.5°C for growth in liquid media, 42°C for plate incubation).

Media: H broth was used for preparation of lysates and in the serum blocking experiments described below. EHA agar was used in plates. Recipes for these media are given by STEINBERG and EDGAR (1962).

Preparation of lysates: Log phase *E. coli* B at 2×10^8 cells/ml were infected with phage at a multiplicity of 3 to 4. The infected cultures were aerated at 30°C (39.5°C for preparation of defective lysates of *ts* mutants) until lysis occurred (about 50 min at 30°C, 30 min at 39.5°C). Cultures were then sterilized with chloroform and centrifuged at low speed to remove unlysed cells and large debris.

Serum blocking protein (SBP) assay: The presence in lysates of antigen which will combine with neutralizing antibody can be detected by the blocking test developed by DE MARS (1955). The test consists of incubating dilute antiserum with antigen for a sufficient length of time for the reaction to go to completion. Residual neutralizing activity is then measured by the inactivation of subsequently added tester phage.

A 30,000-fold dilution of an hyperimmune anti-T4 rabbit serum (S4) was used in these experiments. The undiluted serum had a first-order rate constant (*k*) of 2000/min. Various dilutions of the lysate to be tested were mixed with serum (equal volumes) and incubated at 48°C for 24 hours. Tester phage (*r48* at 2.5×10^6 /ml) were then added and the mixture was reincubated for 2 hours. Samples were then diluted and surviving tester phage assayed on strain F(λ). Plates were incubated at 42°C. These conditions are restrictive for *ts*, *am* and *rII* mutants, thus all plaques formed are due to tester phage and not surviving phage from the lysates under examination. Control experiments showed that inactivation of tester phage by lysate alone (debris) was negligible. Under the conditions employed the antigen-antibody reaction reaches completion after about 8 hours.

Each experiment consists of a series of serial dilutions of lysate to which serum was added and a broth plus serum control. The *k* value of the residual serum in each case was calculated using the broth control as 100 % and assuming exponential inactivation kinetics for neutralization of tester phage. Conditions were such that tester phage survival was always greater than 0.5%.

The type of plot used is shown in Figure 3. Curve A indicates the type of curve obtained with a control lysate. At low concentrations of lysate no antigen is detected and thus the residual antibody activity is 100%. At high concentrations all antibody is complexed and thus the residual activity is 0%. Curve B indicates the shape of curve expected if all antigen species are present in the lysate but in reduced concentration relative to the control. Curve C indicates the result expected if certain antigenic species are absent from the lysate. Curve D indicates the result expected if "altered" antigen is present but with no antigenic species absent.

RESULTS

The blocking activity of defective *am* lysates. The whole serum S4: It had previously been shown (EPSTEIN *et al.* 1963) that conditional lethal mutants defective in the genes 34, 35, 36, 37 and 38 produce no infective progeny upon growth under restrictive conditions although normal numbers of intact virus particles are produced. Preliminary studies indicated that some of these defective lysates were deficient in antigen which will block the neutralizing activity of anti-T4 serum. For more detailed studies of the blocking properties, defective lysates of

FIGURE 3.—A graphical representation of the results of serum blocking tests. The ordinate is the percentage of initial neutralizing activity remaining after incubation with lysate diluted by the factor indicated on the abscissa (a logarithmic scale). The interpretation of the various curves is given in the text.

am mutants defective in these genes were prepared on the restrictive host B. The mutant strains used in this survey are listed in Table 1 and are subsequently referred to by their lysate designation. The *rII* mutant *ra61* or in some cases *rd41* was used as a control since a yield of normal particles are produced which will not however plate on the indicator strain F(λ) used in the blocking experiments.

The ability of these lysates to block the neutralizing activity of whole anti-T4 serum (S4) is shown in Figure 4. Experiments with independently prepared lysates and other anti-T4 sera indicate that the general shapes of the curves are reproducible. It may be seen that, under the conditions employed, tenfold dilutions of the control and the L35 lysates are sufficient to completely block the neutralizing activity of the serum. The rest of the lysates are aberrant in their

TABLE 1
Defective lysates

Infecting phage	Lysate designation	Viable phage/infected cell
<i>ra41</i>	control	200
<i>amb25/ama455*</i>	L34	<0.10
<i>amb252</i>	L35	0.15
<i>ame1†</i>	L36	<0.10
<i>amN52/amb280*</i>	L37	0.15
<i>amb262;amC290</i>	L38	<0.10
<i>amb25/ama455/amb252/amN52/amb252§</i>	LX4E	<0.10
<i>ame198‡;ame62‡</i>	L57	<0.10

* These are double mutants. The single mutations are located near the ends of the gene.

† A new mutant shown to be in gene 36.

‡ New mutants, located in gene 57.

§ A multiple mutant defective in genes 34, 35, 37 and 38.

FIGURE 4.—Blocking of the serum S4 by defective *am* lysates. (○) control-*ra41*; (●) L35; (△) L36; (▲) L34; (▽) L37; (▴) LX4E.

blocking behavior. The lysates with single gene defects (L34, L37, L36) appear to reach “plateaus” suggesting capacity to block only a fraction of the neutralizing antibody. The plateau levels observed for the different defective lysates vary somewhat from experiment to experiment and are sometimes obscured by the “tails” (see below).

These results suggest that in aberrant lysates specific antigens are missing and as a consequence specific neutralizing antibodies directed against these antigens are left unblocked. The multiple mutant lysate (LX4E) appears lacking in ability to block any neutralizing antibody. One would surmise that all antigenic species which can block neutralizing antibody are absent from the lysate of the multiple mutant.

(With all antigenically deficient lysates there are “tails” on the curves at very high lysate concentrations. These tails correspond to about 1% of the activity of the control lysate. In the lysates the input viable phage corresponds to about 1% of the yield of progeny in the control. Thus we believe the “tails” on the curves are probably due to blocking of antibody by the fibers of the input phage not removed from the lysates.)

It was found that an equal mixture of lysates L34 and L37 was able to block all neutralizing activity in the whole serum. This suggests that the antigenic defects of the two lysates are complementary—different antigens being missing in each lysate. To test this notion further, adsorbed sera were prepared. These adsorbed sera were made by incubating a mixture of the whole serum (S4) with lysate at relative concentrations such that the residual neutralizing activity after incubation reflects that found on plateaus. Of necessity these were prepared with somewhat more concentrated sera than that used in the experiments described above so that sufficient activity remained to perform further blocking experiments. These adsorbed sera were serum-lysate mixtures and no attempt to purify the residual antibody was made.

FIGURE 5.—Blocking of the adsorbed serum S4-34 by defective *am* lysates. (O) control-*ra41*; (inverted open triangle) L37; (Δ) *amA455*; (▲) *amN58*; (●) *amB25*.

Adsorbed serum S4-34: A serum adsorbed with lysate L34 was designated S4-34. One might expect that this serum would be specific for antigens lacking in defective lysates of amber mutants defective in gene 34. Figure 5 shows blocking curves for S4-34 with lysates of various *am* mutants. As expected, it was found that the lysate L34 as well as lysates of single *am* mutants located at widely spaced points in this gene (*amB25*, *amN58*, *amA455*) were unable to remove neutralizing antibody from this adsorbed serum. This result indicates that all antibody which will combine with antigens present in the lysates of gene-34 defective mutants were blocked by the adsorption procedure. It further indicates that, within the framework of these experiments, such neutralizing antibody in the whole serum forms *essentially* irreversible complexes with antigen.

Lysates of *am* mutants defective in the other genes behaved in a manner indistinguishable from the control, blocking all neutralizing antibody at concentrations of lysate comparable to the control (L37 is shown). This result shows that these lysates, although in some cases antigenically defective with regard to whole serum, are not defective with regard to the adsorbed serum S4-34. This finding is most readily explained by proposing that an antigen, "A", is absent in all defective lysates of gene-34 *am* mutants but present in normal amounts in lysates of *am* mutants defective in other genes of the tail fiber group. Thus the adsorbed serum S4-34 may be used in blocking tests as a serum specific for antigen A.

The adsorbed serum S4-35: Although the lysate L35 showed blocking activity indistinguishable from the control, the possibility remained that a specific antigen was lacking which could block only a small undetected fraction of the neutralizing activity of the S4 serum. Thus an adsorbed serum was prepared which had about 0.1% residual neutralizing activity. As in the case of S4-34 blocking experiments with the various defective lysates were performed. It was found that

the pattern of blocking with this adsorbed serum was the same as with whole serum (S4). We thus conclude that this adsorption procedure did not enrich the serum for a fraction which was specific for any particular antigen. It appears unlikely that L35 is lacking any antigen which can block neutralizing activity.

Adsorbed serum S4-36: An adsorbed serum was prepared with the lysate L36. In contrast to S4-34, it was found that lysate L37 as well as L36 lacked antigen which would block the neutralizing activity of this serum. L34 and L35 were comparable to the control in their blocking behavior. Thus this adsorbed serum appears to be a specific reagent for a second antigen, "B", which is present in normal amount in L34 and L35, but lacking in L36 and L37.

The adsorbed serum S4-37: As shown in Figure 6, L37 does not block this serum and thus it is specific for the antigens lacking in L37. The lysates L34 and L35 behave like the control and thus contain normal levels of antigens for which this serum is specific. The lysate L36 can only block about 90% of the neutralizing activity of the serum. Since we have already shown that L37 is defective in the production of antigen B, it follows that at least a portion of the residual activity of S4-47 should be directed against antigen B. The fact that L36 can block 90% of the activity of the serum indicates the existence of another antigen "C", probably present in normal amounts in lysate L36, but absent in lysate L37. Thus about 90% of the activity of this serum is against the C antigen and the remaining 10% is directed against the B antigen. Defective lysates of single amber mutants of gene 37 (N52, B280) behave in a manner comparable to L37.

Gene 38 am mutants: L38 lysates (*amB262*, *amC290*) appear to contain low levels of C antigen since they can block S4-37 at high concentrations of lysate (see Figure 6). The curve shape suggests that L38 lysates contain about 10% the normal levels of antigen C (*i.e.*, like curve B, Figure 3, displaced ten-fold from curve A on the abscissa). The same is true of antigen B. Sera adsorbed with L38 lysates are comparable to S4-37 and reveal no new antigens.

FIGURE 6.—Blocking of the adsorbed serum S4-37 by defective *am* lysates. (O) control-*ra41*; (inverted open triangle) L37; (Δ) L36; (▲) L34; (●) L38-*amB262*.

Gene 57 am mutants: Mutants defective in a new gene (57), located between genes 1 and 2 and thus unlinked to the tail fiber cluster have been found to have a defective phenotype similar to the mutants of the tail fiber cluster (EPSTEIN and BOLLE, unpublished). That is, normal numbers of particles are produced under restrictive conditions. The particles are inactive and lack tail fibers. The defective lysates were examined for their blocking behavior and were found to have low levels of all three antigens A, B and C (about 5 to 10%).

Summaries of the results of the experiments so far described are given in Tables 2 and 3. It is clear from the results that, with the exception of gene-38 and 57 mutants, particular antigens are either totally absent or present in normal amounts in the various amber defective lysates. The discrete presence or absence of the antigens in the lysates is in contrast to the results with defective lysates of temperature sensitive mutants in these genes which will now be described.

The blocking activity of defective ts lysates. Gene 34 ts mutants: Lysates of 12 *ts* mutants defective at different sites within gene 34 were prepared and examined for the presence of A antigen by blocking power against serum S4-34. As can be seen from Figure 7 the blocking curves for different defective lysates differ from one another. At one extreme is lysate of mutant *tsN1* which appears indistinguishable from the control, at the other, the lysate of mutant *tsA44* which appears to

TABLE 2

*Blocking behavior of defective amber lysates with various sera**

Sera	Blocking activity of defective lysates				Antigenic specificity of sera
	L34	L35	L36	L37	
S4	PD	N	PD	PD	A,B,C
S4-34	CD	N	N	N	A
S4-35	PD	N	PD	PD	A,B,C
S4-36	N	N	CD	CD	B
S4-37	N	N	PD	CD	B,C
Antigens lacking in lysate	A	None	B	B,C	

* PD—partially defective, CD—completely defective, N—normal.

TABLE 3

Antigenic properties of defective am lysates

Defective in gene	Antigen levels (percent of control)		
	A	B	C
34	0	100	100
35	100	100	100
36	100	0	100
37	100	0	0
38	100	10	10
57	10	10	10

FIGURE 7.—Blocking of the adsorbed serum S4-34 by lysates of *ts* mutants defective in gene 34. The interrupted curve is the control, *ra41*. For clarity only seven *ts* lysates are shown.

contain little if any A antigen. The rest of the lysates give curves which fall in varying degrees between these two. A comparison of these intermediate curves with those of Figure 3 suggest that they do not reflect a deficiency in a specific antigenic class (curve C, Figure 3) nor in most cases low antigen A concentration (curve B, Figure 3). Rather, they differ from the control in slope, suggesting the production of altered antigens which are however capable of binding all antibody species present (curve D, Figure 2). The 12 lysates can be arranged in order as to their blocking power. A striking correlation between blocking power and map position within the gene is observed. The closer the mutation is to the N1 end of the gene the more the blocking activity approaches that of the control. All *ts* lysates were comparable to the control in tests with S4-36 and S4-37. Thus antigens B and C are present at normal levels.

Gene 36 ts mutants: Lysates of three different *ts* mutants of gene 36 were examined for the presence of antigens A, B and C. One (*tsA59*) was normal in its blocking behavior and thus contained normal levels of all three antigens. The remaining lysates, like *amE1* of gene 36, lacked antigen B but had normal levels of antigens A and C.

Gene 37 ts mutants: Lysates of ten different *ts* mutants of gene 37 were examined for their antigenic composition. All but four were similar to amber mutants in this gene, that is, contained normal levels of antigen A but were lacking in antigens B and C. Of the four aberrant mutants, three, *tsL176*, *tsL37* and *tsB32*, located at the "left" end of the gene, lacked antigen B but contained normal levels of antigen C. Although these mutants are antigenically indistinguishable from gene-36 mutants, complementation tests clearly show them to be in gene 37. The lysate of mutant *tsN10*, located at the "right" end of the gene, contained normal levels of all three antigens. Lysates of six mutants located at different sites in the middle of the gene lacked both antigens B and C. Unlike

TABLE 4

Antigenic composition of defective lysates

Gene	Mutants	Antigens		
		A	B	C
34	<i>am</i> (3)*	0†	+‡	+
	<i>ts</i> (12)	var.§	+	+
35	<i>am</i> (1)	+	+	+
36	<i>am</i> (1)	+	0	+
	<i>ts</i> (1)	+	+	+
	<i>ts</i> (2)	+	0	+
37	<i>am</i> (2)	+	0	0
	<i>ts</i> (3)	+	0	+
	<i>ts</i> (6)	+	0	0
	<i>ts</i> (1)	+	+	+
38	<i>am</i> (2)	+	~10%	~10%
57	<i>am</i> (2)	~10%	~10%	~10%

* Number in parentheses indicates the number of different mutants tested.

† 0 indicates no antigens.

‡ + indicates normal antigen levels.

§ var. indicates variable results; see text.

the *ts* mutants of gene 34, *ts* mutants of genes 36 and 37 showed no evidence of blocking curves of intermediate slope; antigen appeared to be either present at normal levels or absent. Gene-38 *ts* mutants have not as yet been found.

A summary of the antigenic structure of the various defective lysates is given in Table 4.

Phage neutralization by adsorbed sera: The kinetics of phage neutralization by the unadsorbed serum S4 is first-order exponential to about 10^{-3} survival. While this is also true of the adsorbed serum S4-34, the adsorbed sera S4-36, and S4-37 give inactivation curves which have a shoulder with an extrapolation number of about five. The shallowness of the shoulder, however, is more compatible with a multi-hit rather than a multi-target inactivation curve. This suggests that for these sera the attachment of about five antibody molecules are required to inactivate the phage. Although the blocking curves were all calculated on the assumption of exponential inactivation kinetics, negligible changes in curve shapes are apparent when the nonexponential nature of the inactivation curves are taken into account.

A gene-34 specific serum evoked by immunization: The following antiserum was prepared by R. HUSKEY. A defective lysate of *am*N52 (gene 37) was prepared and the defective virus particles purified by differential centrifugation. Active particles were removed by adsorption to bacteria followed by low speed centrifugation. A rabbit was given a series of injections of this preparation which contained primarily inactive virus particles. A low titer ($k = 20$) serum was thus obtained and used in blocking experiments. It behaved in all respects like S4-34. That is, lysates of all mutants except those in gene 34 were comparable to the control in blocking neutralizing antibody. Thus all the neutralizing activity of

the serum was directed against antigen A, which we have already shown to be present in normal amounts in gene-37 *am* mutant lysates.

Miscellaneous observations: Our earlier analysis of the phenotypes of various conditional lethal mutants (EPSTEIN, *et al.* 1963) had indicated that only mutants of the tail fiber cluster had impaired antigen production; defects in other morphogenetic steps did not affect tail fiber antigen production. However, only total blocking activity was measured since it was not known that three antigens were involved. Defective lysates of a number of late mutants not concerned with tail fiber synthesis have been examined for the presence of all three antigens. In particular, defective lysates of various multiple amber mutants have been examined—X4a (defective in genes 5, 6, 7 and 8), X4 (10, 11, 12, 17), X2g (2, 4) and X10 (25, 26, 51, 27, 29, 48, 34). All lysates have normal levels of antigens B and C and only X10, which contains a gene-34 amber mutation, is defective in antigen A. Thus these three tail fiber antigens are not dependent upon the activities of these other genes for their formation. In addition, the levels of all three antigens were determined in a lysate of a multiply defective early mutant x5 (defective in genes 41, 42, 43, 44, 45) blocked in DNA synthesis. The cells were lysed with lysozyme at the time when normal lysis would occur. Only 2% of normal levels of antigens A, B, and C were found.

DISCUSSION

The results reported here indicate that the antigenic structure of the tail fibers of phage T4D is complex. At least three characterizable antigens, designated A, B and C, occur in the fiber which combine with neutralizing antibody. The formation of these antigens is controlled by at least five genes.

Studies by FRANKLIN (1961) have also shown the fibers to be antigenically complex and had indicated that a small part of the neutralizing activity of anti-T4 antibody was directed against the sheath rather than the fibers. However, all of the neutralizing activity of the antiserum used in our studies appears to be directed against the antigens A, B and C which are presumably tail fiber components. The defective lysate X4E which contains phage particles with normal sheath and endplates does not block a detectable fraction of neutralizing activity of the serum. Further, an antiserum prepared against phage from a gene-37 amber defective lysate contained only anti-antigen A activity. However, a serum has been evoked with tail-fiberless phage particles purified from a defective lysate of X4E (in which antigens A, B and C are absent). This hyperimmune serum has a low neutralizing activity of which 95% can be blocked with tail-fiberless phage. It would thus appear that neutralizing antibodies do exist, in some sera at least, that are directed toward phage components other than the fibers.

It was found that amber and temperature sensitive mutants from the same gene do not necessarily show, under restrictive conditions, the same antigenic phenotype. Antigens absent in lysates of amber mutants may be present in lysates of temperature sensitive mutants of the same gene. This result is in accord with our notions concerning the nature of amber and temperature sensitive mutations.

SARABHAI, STRETTON and BOLLE (1964) showed that amber mutants are non-sense mutants in the restrictive host, giving rise to only fragments of the gene product. Unlike the amber mutants, temperature sensitive mutants exhibit intragenic complementation (EDGAR, DENHARDT and EPSTEIN 1964; BERSTEIN, EDGAR and DENHARDT 1965) and are most likely missense mutations. It is not surprising that in the cases studied polypeptide fragments are antigenically inactive while proteins with a single amino acid substitution are sometimes antigenically active while functionally inactive.

A "polarity of antigenicity" of the temperature sensitive mutants of gene 34 was found. One might imagine that secondary or tertiary structure is acquired by a protein in a polarized manner as it is being synthesized and that temperature sensitive mutations block this process without however affecting synthesis *per se*. This explanation of the observed polarity appears unlikely since one would expect it to apply to all genes, yet the antigenic polarity is observed with gene-34 but not gene-37 temperature sensitive mutants. A more likely explanation is that the antigenic determinants for phage neutralization on antigen A are determined by the "right" end of the gene. Temperature sensitive mutations at the "left" end of the gene, while rendering the protein inactive, might not disturb its antigenic behavior. Following this line of reasoning the antigenic determinants of antigen C would be controlled by the central portion of gene 37, since temperature sensitive mutations at either end do not interfere with the formation of antigen C.

Defective lysates of gene-57 *am* mutants contain low but significant levels of all three antigens. If it could be shown that these antigens are the direct products of the genes of the tail fiber cluster, it would be possible to consider gene 57 as a "regulator" gene for the genes of the tail fiber cluster. If this were the case, its regulatory action must differ from that of the *i* gene of the lactose operon (JACOB and MONOD 1961) since amber (nonsense) mutations in gene 57 result in a "repression" of the activity of the tail fiber cluster rather than constitutive activity. More analogous are the *i^s* mutations of the *i* gene which, however, are dominant; the gene-57 amber mutants are recessive. Explanations other than regulatory must also be considered. For example, gene 57 may make a product which when combined with antigens A, B and C renders them antigenically active. It is important to note that our method detects only those antigenic sites which when combined with antibody may result in phage inactivation.

The major aim of the studies reported here was toward the elucidation of the roles the various tail-fiber genes play in the formation of tail fibers. Antigen A would appear to be the gene product of gene 34. It is completely absent in defective lysates of gene-34 amber mutants and may be modified in its antigenicity by temperature sensitive mutations of this gene. Mutations in the other genes of the tail fiber cluster do not affect its appearance in defective lysates.

The genetic control of antigens B and C is more complex. Antigens B and C are both absent in defective lysates of amber mutants of gene 37 while only antigen B is lacking in defective lysates of gene-36 amber mutants. Temperature sensitive mutations in these genes may differ from amber mutations in their effects on the production of antigens. However, one rule seems to be obeyed:

antigen B is never present in the absence of antigen C, while antigen C may occur without antigen B. An explanation of these results could be that antigen C is under the control of gene 37 and antigen B, while determined by gene 36, is antigenically dependent upon antigen C. We have no evidence that antigens B and C are the protein products of genes 36 and 37. Antigen B might for example be a hapten made enzymatically by the product of gene 36, or a modified or aggregated form of antigen C.

The fact that the genetic determinants of host range are located in or near gene 37 (R. L. RUSSELL and R. HUSKEY, unpublished) would suggest that the component of the tail fiber concerned with attachment is the product of gene 37 and thus antigen C. If this is true, gene 38 must play a secondary role in the synthesis or modification of antigens B and C. The reason for the presence of low but significant levels of antigens B and C in defective lysates of gene-38 *am* mutants is not known.

These speculations may be summarized as follows. There are two major components of the tail fiber antigen A and antigen BC. Gene 34 controls the synthesis of antigen A. Gene 37 (and possibly gene 38) controls the synthesis of antigen C which is then modified to BC under the control of gene 36. Gene 35 may play a role in the association of these fiber components with the phage particle.

A proper understanding of the nature of the antigenic determinants revealed by this study and their gene control must await studies as to their physical identity. In this regard, at present little is known. DR. F. EISERLING (personal communication) has made an electron microscopic examination of defective lysates of some amber mutants of the tail fiber group. In all cases, intact phage are present which lack tail fibers. Abundant fiber fragments are present but unattached to phage in lysates of all mutants examined except those defective in gene 37, where they are absent. These observations suggest that gene 37 controls the major morphological element of the fiber and that this is not attached to phage when any of the other genes of the tail fiber cluster are nonfunctional. Yet it is puzzling that the gene-34 component is not detected by this method. The co-factor phenotype and antigen A are both determined by this gene, suggesting that it makes a material contribution to the structure of the fiber. The large size of gene 34 would suggest that it would determine a major structural element of the fiber. Clearly, the structure and mode of assembly of the tail fibers will not be completely understood until the elements of the fiber are characterized physically and correlated with the various genes and antigens so far identified.

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SUMMARY

A study of the blocking of antibody neutralization by defective lysates of conditional lethal mutants located in six different genes concerned with tail fiber structure of phage T4 has been made. The results indicate that at least three

distinct tail fiber antigens are involved in the neutralization of phage by antibody and that two of these antigens are under multigenic control. Amber and temperature sensitive mutants in the same gene may differ in their antigenic phenotypes. A new gene unlinked to the tail fiber gene cluster was found to specifically affect the production of all three antigens. A regulatory role for this gene is not ruled out.

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