Genetic Studies on Capsid-Length Determination in Bacteriophage T4

I. Isolation and Partial Characterization of Second-Site Revertants of a Gene 23 Mutation Affecting Capsid Length

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The T4 mutation *ptg19-80* affects the mechanism of capsid-length determination. It is located in gene 23, which encodes the major structural protein of the capsid. The mutation results in the production of abnormal-length capsids in high frequencies. This paper describes the isolation and partial characterization of second-site revertants of *ptg19-80*. In the course of their analysis, it was discovered that *ptg19-80* is itself a double mutation consisting of a gene 23 mutation (*ptg19-80c*), which causes the morphogenetic defect, and a suppressor mutation located near the lysozyme gene. Phenotypic characterization of nine pseudo-wild-type revertants of this double mutation revealed that these revertants all produced lower frequencies of abnormal capsids than did *ptg19-80*. Seven of these revertants were shown to contain two suppressor mutations, one mapping in or near gene 22 and one mapping in or near gene 24. Both mutations were required for suppression. These suppressors displayed no discernible phenotype in the absence of *ptg19-80c*.

The structure and assembly pathway of the capsid of bacteriophage T4 are now fairly well understood. Freeze-fracture electron microscopy (4) and optical diffraction of electron micrographs (1) have shown that the T4 capsid has the shape of a prolate icosahedron elongated along one axis of fivefold symmetry. Although the details of the capsid assembly process remain to be determined, it seems clear that the mature capsid is derived from a capsid precursor termed a prehead or tau-particle (3). During formation of the prehead, an icosahedral shell of gp23 (the product of gene 23), which is the main essential structural component of the mature capsid (14), is assembled around a scaffold-like structure termed the morphogenetic core. The principal essential protein of this core is gp22, although several other proteins are also components of the core structure (18, 28). In the process of capsid maturation, the core is proteolytically degraded (19).

The mechanism which regulates the length of the T4 capsid remains obscure. Two types of models have heretofore been suggested. One type postulates that capsid length is determined through specific protein-protein interactions involving the components of the prehead. The

principal models of this type are the vernier model proposed by Paulson et al. (24) and the cumulated-strain model of Kellenberger (15, 16). A second type of model, termed the kinetic model, has been described by Showe and Onorato (27). They propose that capsid length is regulated by the relative rates of assembly of the morphogenetic core and the capsid shell, which implies that length determination is controlled by the intracellular concentrations of the protein components of these structures.

This and the accompanying paper (10) describe the results of genetic studies aimed at identifying and characterizing protein-protein interactions involved in capsid-length determination to test the current models of length determination. The approach employed was to isolate second-site revertants of a mutation (*ptg19-80*) in gene 23 which results in the production of abnormal-length capsids. A number of such revertants were found which contained suppressor mutations located outside gene 23. Analysis of these suppressors presented in this paper yielded the following, somewhat surprising, results: (i) *ptg19-80*, previously described as a single mutation (8, 9), is itself a double mutation consisting of a gene 23 mutation which affects capsid-length determination and a genetically distant mutation which partially suppresses the gene 23 lesion, and (ii) in seven revertants of *ptg19-80* (itself a double mutation), suppression

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TABLE 1. Phage strains^a

Amber mutation	Gene
B17	23
E506	23
E1270	23
E1236	23
N90 ^b	21
E209	22
E355	24
lys8-82	e
N51	2
B23	7
S52	25
N54	31
N81	41
E506/E1270	23/23
E209/E506/E1270/E355	22/23/23/24
N90/E209/E506/E1270/E355	21/22/23/23/24
N90/E209/E506/E1236	21/22/23/23
lys8-82/E506/E1270	e/23/23
E506/E1270/N54	23/23/31
E506/E1270/N81	23/23/41

^a Mutants were obtained from the collection of A. H. Doermann. Most of the single amber mutations were originally obtained from the California Institute of Technology Collection. Most of the multiple amber mutations were constructed in the course of these studies.

^b The N90 mutation failed to grow on *E. coli* B and grew well on *E. coli* CR63. However, it also failed to grow on *supD* derivatives of *E. coli* B (22). Therefore, it may not be a chain terminator mutation. For purposes of genetic mapping, this question is moot.

is brought about by two mutations (one mapping in or near gene 22 and one mapping in or near gene 24). Both mutations must be present for suppression.

This work provides evidence for particular protein-protein interactions involved in capsid-length determination. The accompanying paper (10) further these observations by making a more critical determination of the genic location of the suppressor mutations and by studying the mechanism of suppressor action in a manner which provides some insight into the validity of current models of T4 capsid-length determination.

MATERIALS AND METHODS

Bacteria and phage. Two strains of *Escherichia coli* were employed in this work, B and CR63. *E. coli* B is nonpermissive for growth of phage carrying amber mutations, whereas *E. coli* CR63 (*supD*) efficiently suppresses all of the amber mutants used. T4D makes large, regular plaques on *E. coli* B. On *E. coli* CR63, plaques are smaller and less uniform. Hence, *E. coli* B was used as the plating bacterium in all instances in

which plaque morphologies were compared. Plaque assays were typically performed with log-phase plating bacteria as described by Krieg (17).

Phage strains used in this study were all variants of T4D. In addition to T4D wild type and the mutant strain *ptg*19-80 (8), a number of single and multiple amber mutants were employed. These amber strains are listed in Table 1. Approximate map locations of the mutations are shown in Fig. 1.

Media. Bacterial cultures were grown on either Hershey broth (12) or M9AA medium (8). Plate agar (bottom-layer agar) and soft agar (top-layer agar) employed in these experiments were as described by Chase and Doermann (6).

Preparation of phage stocks. (i) **Method A.** Stocks of single and multiple amber mutants were grown from single young (4- to 6-h) plaques. Bacteria for these stocks were prepared by growing a 400× dilution of a saturated culture of *E. coli* CR63 in Hershey broth at 30°C for 2.5 h, giving a titer of about 2×10^7 bacteria per ml. A single young plaque was added to 20 to 30 ml of bacterial culture and shaken overnight (16 h) on a rotary shaker at 30°C. These stocks were lysed by CHCl_3 , centrifuged to remove cellular debris, adjusted to pH 6.8, and stored over CHCl_3 . Some stocks of *ptg* mutants were prepared in a similar manner, although bacterium B was employed instead of CR63.

(ii) **Method B.** When the phenotypes of lysates (i.e., types and frequencies of abnormal capsids) were to be quantitatively measured and compared, stocks were prepared by the following variation of the more precisely controlled lysis inhibition procedure of Doermann et al. (8). A saturated overnight culture of strain B was grown (at 37°C in M9AA medium) to a titer of 2

FIG. 1. Genetic maps of some T4 genes and mutations. (A) Approximate locations of pertinent T4 genes. This map is according to Wood and Revel (30). (B) Order of some amber mutations in the region of genes 21 through 24. The distances are not drawn to scale.

$\times 10^8$ to 4×10^8 cells per ml. This culture was infected at 37°C at a multiplicity of infection of 5 phage particles per cell and then superinfected 15 min later, again at a multiplicity of infection of 5. Under these conditions, lysis inhibition results and most of the infected bacteria are still intact at 120 min postinfection. At this point, cultures were either lysed by addition of CHCl_3 or first chilled on ice and then sedimented (5,000 rpm for 10 min in a Sorvall RC-2B centrifuge with a GSA rotor) to pellet the infected bacteria. The pellet of infected cells was resuspended in a small volume of M9 salt solution (M9AA medium lacking amino acids and glucose) and lysed with CHCl_3 . This procedure results in concentration of the lysate and produces stocks of unusually high titers. Both concentrated and unconcentrated stocks were treated with DNase and centrifuged at a low speed (4,000 rpm for 20 min in a Sorvall RC-2B centrifuge with an SS34 rotor) to remove cellular debris.

Titration of non-plaque-forming phage particles. Phage particles with shorter than normal capsids contain only partial copies of the T4 genome. Therefore, a bacterium which is singly infected by such a particle does not yield a burst of phage. Petite particles can, however, efficiently adsorb to bacteria and inject their DNA. This results in the death of the host, even in the absence of phage particle production. The killing ability can be used to assay the total number of particles in a lysate. The distribution of phage adsorbed to a bacterial population can be well approximated by the Poisson distribution at multiplicities of infection of <5 phage per bacterium (11). Therefore, actual multiplicities of infection were determined as follows. Equal volumes of *E. coli* B at 2×10^8 cells and a phage suspension with an estimated particle titer of 4×10^8 to 6×10^8 were mixed and shaken at 30°C for 10 min, at which time $>90\%$ of the phage was adsorbed. The mixture was then diluted 50-fold into ice-chilled Hershey broth to slow intracellular phage growth and assayed for bacterial colony-forming units. From the observed fraction of surviving bacteria, the actual multiplicity of infection was calculated with the formula from the Poisson distribution.

Mutagenesis. The production of mutagenized phage stocks with 5-bromo-2'-deoxyuridine was performed as described by Paietta et al. (23).

UV irradiation of phage. Phage samples to be UV irradiated were diluted to the desired titer in UV-transparent M9 salt solution (i.e., M9AA medium lacking glucose and amino acids) supplemented with gelatin at 10 or 20 $\mu\text{g}/\text{ml}$. Samples were placed in watch glasses 1 m from the UV source, a 15-W Germicidal Lamp (General Electric Co.) powered by a constant-voltage transformer (Sola Electric, Div. General Signal). Phage survival at relatively low UV doses was assayed directly. At high UV doses, the average number of lethal events per phage genome (PLH) was determined by extrapolating from the measured phage survival at lower doses, where the number of lethal events per unit time of irradiation is given by the slope of the survival curve. Assays of irradiated phage were always conducted under yellow light to prevent photo-reactivation.

Electron microscopy. For electron microscopy examination, phage samples were diluted to 10^{11} particles per ml in M9 salt solution. Specimens were prepared by negative staining with uranyl acetate as

described previously (8). However, the pH of the saturated uranyl acetate was adjusted to 3.5 with HCl.

Crosses. Genetic crosses involving amber mutants were performed in CR63. If no amber mutants were involved, *E. coli* B was employed. Bacteria were grown at 30°C in H broth. A 1,000-fold dilution of a saturated overnight culture was grown for 2.5 h to a titer of 10^7 . These cells were pelleted and resuspended to a titer of 2×10^8 in ice-chilled H broth.

A sample of this bacterial suspension was added to an equal volume of a mixture of the two phage (each at an adsorbed multiplicity of infection of 5). This mixture of bacteria and phage, the adsorption culture, was shaken in a shaker-water bath at 30°C . At 8 min after mixing, a sample was taken from the adsorption culture and diluted 20-fold into H broth containing CHCl_3 . CHCl_3 treatment causes destruction of infected cells before production of phage particles. This sample thus yielded an assay of unadsorbed phage. Immediately after obtaining this sample, anti-T4 antiserum was added to the adsorption culture to inactivate the unadsorbed particles. At 15 min after mixing (at which time the titer of unadsorbed phage had been reduced by a factor of 10^3), the adsorption culture was diluted 5×10^4 , and the infected bacteria were immediately assayed. Many crosses involved one parent that had been UV irradiated. The same general procedures were followed, but antiserum was generally omitted and unadsorbed phage were not usually assayed. These crosses were always carried out under yellow light.

RESULTS

Isolation of revertants. The aim of this work was to isolate mutations that suppress the morphogenetic lesion present in *ptg19-80*. The mutation *ptg19-80* makes plaques which are smaller than wild-type plaques. In addition, it produces ($\sim 80\%$) particles with shorter-than-normal, but prolate, capsids. These particles are termed intermediates. It also produces some particles ($\sim 10\%$) with even shorter capsids which appear to be regular icosahedra. These particles are called isometrics. Isometrics and intermediates are both referred to as petite particles. Normal-length phage particles are also produced ($\sim 10\%$). In addition, at a low frequency ($\sim 1\%$), giant phage particles, which have capsids ranging in length from slightly greater than normal to more than 10 times normal length, are produced. More than 50 similar mutations have been isolated by selection for the ability to make giant particles (23; A. H. Doermann, unpublished data). These mutations are termed *ptg* (petite and giant former) mutations. Precise genetic mapping has shown that the sites where these mutations occur comprise three clusters within gene 23 (9; Doermann, unpublished data).

Selection for reversion of this phenotype could not be carried out directly. However, it was known that all *ptg* mutations form plaques which are smaller than wild-type plaques and which are readily distinguished from wild-type

plaques when plated on *E. coli* B, owing almost certainly to the production of high frequencies of non-plaque-forming particles (petite particles) by the *ptg* mutations. Therefore, the approach employed was to screen *ptg*19-80 for reversion of the plaque morphology phenotype as a probe for suppression of the production of petite particles. The results eventually demonstrated that relative plaque size is a good indicator of the frequency of petite particles produced; i.e., mutants that produce relatively large plaques produce relatively low frequencies of petite particles, whereas mutants which produce smaller plaques produce relatively high frequencies of petite particles.

It has been noticed that many *ptg* mutations, including 19-80, give rise to revertants (of plaque morphology) at high frequencies, owing probably to a selective advantage of wild-type over *ptg* mutations. The frequency of wild type among lysates of *ptg*19-80 ranged from $<10^{-4}$ to $>10^{-2}$. A total of 27 spontaneous, wild-type-appearing revertants of *ptg*19-80 were picked. Stocks of these revertants were backcrossed to wild type or a gene-23 double amber mutation (E506/E1270) to test for segregation of *ptg*19-80. In no instance was segregation of the *ptg*19-80 plaque type observed. These wild-type revertants were, therefore, either true back mutations at the site of the 19-80 mutation or possibly second-site revertants which contained a suppressor mutation very tightly linked to the 19-80 site.

One spontaneous revertant, designated 19-80/F2LG, was found which was pseudo-wild type in plaque morphology, i.e., intermediate in size between *ptg*19-80 and wild type. When backcrossed, this revertant segregated the 19-80 plaque type and, in addition, a new type which was intermediate in size between 19-80 and 19-80/F2LG. Further searches for spontaneous, pseudo-wild-type revertants proved fruitless, although 2×10^5 plaques were screened. It was then decided to employ a mutagen to increase the frequency of pseudo-wild-type revertants, which appeared to constitute a more interesting class than the wild-type-appearing revertants. A stock of *ptg*19-80 was subcultured to produce five stocks of 5-bromo-2'-deoxyuridine-substituted phage, as described in Materials and Methods. These stocks were plated on *E. coli* B, and approximately 10^5 plaques from each stock were examined. A total of 25 pseudo-wild-type plaques were observed. These were picked, and stocks of these were backcrossed. The 19-80 plaque type was observed to segregate in each of the backcrosses. Of these 25 revertants, 8, along with the spontaneous revertant 19-80/F2LG, were chosen for further characterization. These eight were chosen as being either phenotypically

distinct (in plaque morphology) or of independent mutational origin.

Phenotypic characterization of second-site revertants of *ptg*19-80. The frequencies of normal and petite phage produced by the revertants and 19-80 were determined by measuring the titers of plaque-forming and non-plaque-forming particles. The titer of particles capable of killing an *E. coli* cell, the bacteria killing ability (BKA) titer, can be assayed by the method described in Materials and Methods. This titer can be compared with the titer of plaque-forming particles. The BKA-plaque-forming particle ratio, termed the BKA ratio, equals the ratio of total phage particles to plaque-forming particles. The reciprocal of this term is the fraction of plaque-forming particles present in the sample. Giant phage particles (as well as normal-length particles) are infectious and form plaques upon single infection (2, 8). However, giant particles are usually not frequent enough in *ptg*19-80 lysates to constitute a significant fraction of the plaque-forming population; i.e., under normal circumstances, no significant errors are introduced by equating normal-length particles and plaque-forming particles.

BKA ratios of stocks of 19-80 and the nine revertants are shown in Table 2. These stocks were all made by method B, as described in Materials and Methods. It can readily be seen that all of the revertants differed significantly from 19-80 (and wild type) in the frequencies of petite and normal particles produced, although the total number of particles produced did not differ significantly (data not shown). All revertants showed increased frequencies of normal particles and decreased frequencies of petite particles. However, all revertants still produced a significant fraction of petite particles. The revertants seemed to be pseudo-revertants, not

TABLE 2. BKA ratios of 19-80 and revertants

Mutation	Ratio	
	BKA ^a	1/BKA ^b
19-80	8.3	0.12
19-80/BUR1	3.4	0.29
19-80/BUR2	3.6	0.28
19-80/BUR6	3.2	0.31
19-80/BURA1	1.4	0.71
19-80/BURA2	2.5	0.40
19-80/BURB2	3.0	0.33
19-80/BURC1	3.9	0.26
19-80/BURD1	2.0	0.50
19-80/F2LG	1.3	0.77
Wild type	0.95	1.05

^a BKA values shown are the average of two to four determinations.

^b 1/BKA is equal to the frequency of plaque-forming particles.

only in plaque morphology, but also with respect to production of petite-phage particles.

The production of particles with longer-than-normal capsids, or giant phage particles, was assayed by measuring the kinetics of phage inactivation by UV irradiation. It is known that giant particles are much more resistant to UV inactivation than are normal-length particles (8), presumably owing to the fact that giant particles encapsidate and inject a multiple-copy concatemer of the T4 genome, allowing multiplicity reactivation (13, 20) to occur between undamaged portions of the multiple genomes and thereby producing progeny.

Samples of wild type and 19-80 and its revertants were irradiated as described in Materials and Methods. Survivors were assayed by plating on *E. coli* B. The number of irradiated phage particles plated per plate was always $<5 \times 10^{-4}$ per bacterium. This precaution was taken to avoid plaque formation via multiplicity reactivation arising in multiply infected cells (20, 21). Experimental work of Harm (13) has shown that, under these plating conditions, the contribution of multiplicity reactivation to the measurement of survival is negligible. The biphasic survival curves shown in Fig. 2 demonstrate that all of the revertants except 19-80/F2LG still produced some giant particles. The survival curve of 19-80/F2LG (Fig. 2C) was indistinguishable from the wild-type curve. From this result, it was concluded that 19-80/F2LG no longer produced giant phage. Electron microscopy examination of 19-80/F2LG also failed to detect the presence of giant particles.

***ptg*19-80 is a double mutation.** Before attempting to describe the genetics of the revertants of *ptg*19-80, it is necessary to redefine *ptg*19-80. As originally described (8, 9), *ptg*19-80 behaved as a single mutation. However, during the investigation of the second-site revertants, it was discovered that *ptg*19-80 consists of two components which are, in effect, a *ptg* mutation (located in gene 23 at the site to which 19-80 was originally mapped) and a suppressor mutation which partially reverts the *ptg* phenotype. This suppressor maps near the lysozyme locus (gene *e*).

The crucial observation leading to this discovery was made by Doermann and Pao (unpublished data), who isolated about 50 independent *ptg* mutations and mapped them via plaque morphology to gene 23. They were interested in demonstrating the genetic purity of these mutations and eliminating any extraneous mutations that might be found to influence plaque morphology. By utilizing the detailed amber map of gene 23 that is available (5; Doermann, unpublished data), a very powerful technique was devised to "clean up" these gene 23 mutations (i.e., to detect and, if found, eliminate extraneous muta-

tions). The procedure is as follows. The *ptg* mutant is UV irradiated to a survival of e^{-100} . These heavily damaged phage are crossed to the gene 23 amber mutation nearest to its map location or to a closely linked double amber mutation that spans the *ptg* locus. This cross is performed in *E. coli* CR63 (*supD*), and thus, no gene function need be provided by the irradiated parent. Replication of UV-irradiated chromosomes is severely impaired. Therefore, any segment of the parental *ptg* genome can be represented in the progeny of the cross only through very rare recombination events between the irradiated genomes and the unirradiated genomes of the amber parent. When the progeny of such a cross are plated on *E. coli* B (*sup*⁰), only those progeny which have acquired the *am*⁺ allele(s) via recombination will make

FIG. 2. UV survival curves of 19-80 and revertants. Phage samples were irradiated and assayed for survival of PFU as described in the text. An experiment with a wild-type (wt) control was performed (A). The line generated by these data points is drawn as a reference line in all of the panels (B through F). The wild-type data points are not shown in each panel, however. (A) Wild type (star) and 19-80 (●); (B) 19-80/BUR6 (●) and 19-80/BURD1 (*); (C) 19-80/BUR2 (*) and 19-80/F2LG (●); (D) 19-80/BURA1 (*) and 19-80/BURA2 (●); (E) 19-80/BURC1 (*) and 19-80/BURB2 (●); (F) 19-80/BURI (*).

FIG. 3. Plaque morphologies of *ptg*19-80 and *ptg*19-80c. Phage were plated on *E. coli* B, using plates poured to a uniform thickness, and grown at 37°C for 24 h. (A) *ptg*19-80 (19-80c/sup-or); (B) *ptg*19-80c; (C) wild type.

plaques. The frequency of *am*⁺ progeny in such a cross depends on UV dose and parental multiplicities of infection. A typical frequency might be 10^{-4} to 10^{-5} . Any other unlinked locus brought into the cross by the UV-irradiated parent will be present among the progeny of the cross at a similar frequency. It was observed that the frequency of *am*⁺ progeny from a cross of a double amber mutation (consisting of two genetically distant amber mutations) with irradiated wild type was roughly the product of the frequencies of *am*⁺ progeny from the crosses of each single amber mutation to irradiated wild type (unpublished observations). This means that the amber-plus progeny of this cross were nearly isogenic to the amber parent, differing only at a portion of the genome around the amber locus. The *am*⁺ progeny derived that segment of their genomes from the irradiated *ptg* parent. Experimental evidence showed that this segment was, on the average, quite small. A fraction of the time, the site of the *ptg* mutation was included in this segment. This phenomenon is termed corescue of the *am*⁺ and *ptg* alleles. The frequency of corescue depends on UV dose and the physical distance between the two loci. If the *ptg* mutation has a secondary mutation which affects plaque morphology, the segregation of the *ptg* mutation from this mutation may be observed among the *am*⁺ progeny of such a cross, even if the second mutation is closely linked.

When irradiated *ptg*19-80 [19-80(irr)] was crossed by *am*E506/*am*E1270, the *am*⁺ progeny displayed a new, smaller plaque morphology, termed 19-80c (19-80 clean). These plaque morphologies are shown in Fig. 3.

Phenotype of 19-80c. The production of petite and giant phage by 19-80c was measured. A stock of 19-80c was prepared by method B, and the BKA ratio of this stock was assayed. In two experiments, the BKA values measured were 30.3 and 28.0. These values corresponded to

frequencies of plaque-forming particles of 0.033 and 0.036, respectively, as compared with a frequency of 0.12 for 19-80. The phage particle yield per cell of this stock was not markedly different from that of 19-80. From these results, it was concluded that 19-80 is composed of the *ptg* mutation, 19-80c, plus a suppressor mutation that not only affects plaque morphology but also partially suppresses the morphogenetic defect of 19-80c. The mutation 19-80c still produces a significant quantity of giant particles, as measured by kinetics of inactivation by UV.

Mapping the suppressor of 19-80c. Some preliminary observations indicated that the suppressor, termed sup-or (suppressor-original), was genetically distant from 19-80c and that sup-or was, by itself, not discernibly different from wild type in plaque morphology or any other observable phenotype. Therefore, to map this mutation, 19-80 (sup-or/19-80c) was crossed with a series of amber mutations in various genes around the T4 map. The frequency of segregation of the 19-80c plaque type observed in platings of progeny on *sup*⁰ bacteria should be a function of the distance between sup-or and a given amber locus. Specifically, a cross between 19-80 and an amber mutation very near sup-or should yield a low frequency of 19-80c plaques among progeny plated on a nonsuppressing host. The crosses performed are shown in Table 3, along with the frequencies of 19-80c segregants observed in platings of the progeny on *E. coli* B. These results suggested that sup-or is located in the vicinity of the lysozyme gene (e).

A stronger demonstration of this linkage could be made by a corescue experiment that is a variation of the clean-up technique already described. For this experiment, a series of triple amber strains was constructed. These strains all carried *am*E506 and *am*E1270 plus a third amber mutation, X. These triple amber mutations were crossed with 19-80(irr) at several doses of irra-

TABLE 3. Crosses of 19-80 with various amber mutations: frequencies of 19-80c progeny^a

Amber mutation	Gene	Frequency of 19-80c progeny ^b
lys8-82	e	0.05, 0.05
N51	2	0.12
B23	7	0.19, 0.18, 0.20
B17	23	0.17
E1236	23	0.17
S52	25	0.25, 0.21, 0.27
N54	31	0.22, 0.18
N81	41	0.15

^a Crosses were performed as described in the text.^b Fraction of *am*⁺ progeny which were 19-80c. Each number is the result of an independent cross.

diation. The presence of the two amber mutations in gene 23 ensured that, when progenies from these crosses were plated on *sup*⁰ bacteria, virtually all plaque-formers would carry the *ptg19-80c* mutation. The plus allele of *amX* was also obligatory for plaque formation. If *sup-or* had been corescued with the plus allele of *amX*, then the plaque formed would have the morphology characteristic of the original *ptg19-80*. If *sup-or* was not corescued, then the plaque would be of the 19-80c type. The frequency of 19-80 plaques equals the frequency of corescue of the plus allele of *amX* and *sup-or*; this is a measure of the distance separating the two loci.

The crosses made and their results are listed in Table 4. Clearly, the frequency of corescue of *sup-or* and the plus allele of the lysozyme amber mutation was greater than that of corescue of *sup-or* and any one of the other loci. There appeared to be no correlation between the presence of the plus allele of the other amber mutations and the *sup-or* mutation; the frequency of 19-80 plaques in these crosses was about the same as the frequency of 19-80 plaques observed in control crosses of 19-80(irr) with the gene 23 double mutation *amE506/amE1270*.

These results confirmed and strengthened the conclusion from the more conventional mapping experiments; i.e., *sup-or* is located in the vicinity of gene e. Only two genes located in this region are known to be involved in capsid assembly. These genes are IPII and IPIII, which encode nonessential components of the morphogenetic core (25).

Phenotype of *sup-or*. *Sup-or* was isolated free of 19-80c from a cross of *ptg19-80* (*sup-or*/19-80c) with wild type. That this stock was indeed *sup-or* was demonstrated by crosses with 19-80 and 19-80c. When *sup-or* was crossed with 19-80c, approximately 15% of the progeny were of the 19-80 plaque type. Conversely, in crosses with 19-80, no segregation of the 19-80c plaque morphology was observed. A stock of *sup-or*

prepared by method B was found to have a BKA ratio of 1.05. The UV survival curve of *sup-or* was indistinguishable from the wild-type curve. Thus, it appeared that the suppressor of *ptg19-80c* had, of itself, no obvious morphogenetic effects.

Genetic analysis of seven second-site revertants.

This discovery of the true nature of the mutation *ptg19-80* suggested that a paradox existed in one of the initial observations on the pseudo-wild-type revertants of 19-80. The revertants were originally classified as second-site revertants because they segregated the 19-80 plaque type when backcrossed to wild type or amber mutations in gene 23. The paradox is that, in these crosses, the segregation of 19-80c should have occurred (because *sup-or* is quite distant), but this event was not observed. One possible explanation of the failure to detect 19-80c segregation is simply that the 19-80 and 19-80c plaques were not distinguished (in the early stages) when these crosses were scored. However, subsequent reexamination of the progeny of these crosses indicated that, indeed, the 19-80c plaque type segregated only rarely in crosses involving all revertants except 19-80/BUR6 and 19-80/BURC1. Crosses of these two revertants with wild-type revertants did segregate appreciable frequencies (10 to 12%) of 19-80c plaques. These two revertants proved intractable to genetic analysis. The genetic analysis of the remaining seven revertants will now be considered.

The genetics of these seven revertants proved to be qualitatively quite similar. Reversion of the 19-80 parent (19-80c/*sup-or*) was found to be brought about by two additional mutations. These two new mutations (termed x and y) were found to be closely linked to, and flanking, the 19-80c locus. The resolution of the paradox lay in the realization that phage of the genotype x/

TABLE 4. Frequencies of 19-80 plaque type among *am*⁺ progeny in corescue crosses^a

Amber parent (gene)	Frequency with following UV dose (PLH) of 19-80 parent ^b :		
	6.6	13.2	24
E506/E1270 (23/23)	NT ^c	0.05	0.02
E506/E1270/N54 (23/23/31)	0.24	0.07	0.03
E506/E1270/N81 (23/23/41)	0.20	0.05	0.01
lys8-82/E506/E1270 (e/23/23)	0.65	0.45	0.19

^a Crosses of amber mutations by 19-80(irr) were performed as described in the text.^b A dose of *m* PLH is defined as the dose which results in a frequency of survival of *e*^{-m}. Numbers are the frequencies of 19-80 plaques among the *am*⁺ progeny of each cross.^c NT, Not tested.

19-80c/y (wild type for sup-or) gave rise to plaques which were very similar to the 19-80 plaque type produced by phage of the genotype 19-80c/sup-or. Therefore, in crosses of revertant (sup-or/x/19-80c/y) with wild type, the closely linked mutations x, 19-80c, and y frequently cosegregated from the distant sup-or locus and gave rise to the 19-80-type plaques observed among the progeny of these crosses. The experimental basis for this model of the genetic structure of the revertants will now be presented.

The 19-80-like segregants from crosses of wild type with revertants were picked, and stocks were made. These stocks were given the designation *ps*19-80 (pseudo 19-80). The term *ps*19-80 was employed in the belief (subsequently vindicated) that these plaques, although morphologically identical to 19-80, were produced by phage of a genotype other than that of 19-80 (19-80c/sup-or). A number-letter combination added to this symbol denoted the particular revertant from which a given *ps*19-80 was derived. For example, from the progeny of the cross of revertant 19-80/BURB2 with wild type, a 19-80-appearing plaque was picked and cultured to yield a stock designated *ps*19-80(B2). The intermediate plaque type, which segregated from the revertant 19-80/F2LG, was also picked and cultured. For consistency, it was designated *ps*19-80(F2LG). Photographs of all *ps*19-80 plaque morphologies are shown in Fig. 4 along with the corresponding revertants of 19-80.

The *ps*19-80 mutations were crossed with sup-or. Each cross produced a high frequency (~15%) of the initial revertant from which the particular *ps*19-80 strain involved in the cross was isolated. These results indicated that each revertant required sup-or for maximal suppression and that each revertant was comprised of sup-or plus whatever mutation(s) constituted the *ps*19-80 genotype.

***ps*19-80 equals 19-80c plus additional suppressor mutations.** The *ps*19-80 mutations could arise from pseudo-reversion at the original 19-80c locus, or they might be combinations of 19-80c with another mutation(s), resulting in the phenotypic differences between 19-80c/sup-or and the revertants. To critically test the revertants for the presence of the original 19-80c mutation, the UV clean-up technique was employed. Each revertant was irradiated to three UV doses, 24, 48, and 96 PLH. The irradiated revertants were crossed to the double amber mutation in gene 23, *amE506/amE1270*, and the progeny of these crosses were plated on *E. coli* B (*sup*⁰). If the revertants, and consequently the *ps*19-80 mutations, contained 19-80c, then these crosses should have segregated 19-80c plaques when plated on *E. coli* B. If the revertants had a new mutation at or very near the 19-80c site, then

primarily *ps*19-80 plaques should have been observed in the *am*⁺ progeny, whereas 19-80c plaques should have occurred rarely or not at all. The results of these crosses are shown in Table 5. At low UV doses, both 19-80c and *ps*19-

TABLE 5. Crosses of *amE506/amE1270* with irradiated revertants^a

Revertant and UV dose (PLH) ^b	Frequency among <i>am</i> ⁺ ^c		Frequency of <i>am</i> ⁺ in progeny ^d
	19-80c	<i>ps</i> 19-80	
19-80/BUR1			
24	0.29	0.27	8.19×10^{-3}
48	0.51	0.04	1.23×10^{-3}
96	0.86	<0.01	2.53×10^{-4}
19-80/BUR2			
24	0.26	0.37	6.51×10^{-3}
48	0.61	0.08	8.91×10^{-4}
96	0.74	0.01	1.85×10^{-4}
19-80/BURA1			
24	0.41	0.32	2.77×10^{-3}
48	0.76	0.05	2.67×10^{-4}
96	0.78	0.03	3.94×10^{-5}
19-80/BURA2			
24	0.31	0.28	3.06×10^{-3}
48	0.73	0.06	2.89×10^{-4}
96	0.89	<0.01	5.92×10^{-5}
19-80/BURB2			
24	0.64	0.32	1.61×10^{-3}
48	0.97	0.03	3.68×10^{-4}
96	0.98	<0.01	8.17×10^{-5}
19-80/BURD1			
24	0.52	0.24	2.68×10^{-3}
48	0.66	0.08	2.63×10^{-4}
96	0.83	0.02	5.88×10^{-5}
19-80/F2LG			
24	0.47	0.48	3.86×10^{-3}
48	0.88	0.10	6.30×10^{-4}
96	0.95	0.03	1.05×10^{-4}

^a Crosses were performed as described in the text.

^b UV dose was as calculated from the fraction of survivors; i.e., survival of e^{-m} defined a dose of m PLH. At high doses (where phage survival cannot be directly assayed), the number of PLH was determined by extrapolating from measured survival at lower doses.

^c The sum of these numbers is often <1.00. This is because, in several crosses, a new plaque type somewhat smaller than 19-80c was observed to segregate. This plaque type is not pertinent to the question considered in this table, namely, the presence or absence of the 19-80c mutation. It will be presently considered in a more appropriate context.

^d Variation in the frequency of *am*⁺ progeny among revertants at comparable doses probably reflects differences in the multiplicity of parental phage owing to variation in bacterial titers.

FIG. 4. Plaque morphologies of revertants of *ptg19-80* and the related *ps19-80* strains. Phage were plated on *E. coli* B, using plates poured to a uniform thickness, and grown at 37°C for 48 h. (A) Wild type; (B) *ptg19-80*; (C) 19-80/BUR1; (D) *ps19-80*(R1); (E) 19-80/BUR2; (F) *ps19-80*(R2); (G) 19-80/BURA1; (H) *ps19-80*(A1); (I) 19-80/BURA2; (J) *ps19-80*(A2); (K) 19-80/BURB2; (L) *ps19-80*(B2); (M) 19-80/BURD1; (N) *ps19-80*(D1); (O) 19-80/F2LG; (P) *ps19-80*(F2LG).

80 were observed among the *am*⁺ progeny. At higher doses, where the average length of the rescued segment of the irradiated genome was decreased, the frequency of the *ps19-80* type

decreased. At a UV dose of 96 PLH, <4% of the *am*⁺ progeny of each cross were *ps19-80*. At this high dose, the frequency of 19-80c among the *am*⁺ progeny ranged from 0.74 to 0.99. These

data showed that all of these revertants still retained the original 19-80c mutation. Thus, the *ps19-80* mutations are composed of 19-80c plus an additional mutation(s).

Mapping the mutations that compose *ps19-80*. The occurrence of significant frequencies (0.24 to 0.48) of the *ps19-80* plaque type among the *am*⁺ progeny of the crosses between E506/E1270 and the revertants irradiated to 24 PLH (see Table 5) demonstrated that the mutation(s) which converts 19-80c to *ps19-80* must be linked to 19-80c; that is, 19-80c and the mutation(s), henceforth termed S mutations, were frequently cosegregated at this dose. The proximity of the S mutations to 19-80c afforded an approach to mapping the S mutations, despite the fact that no phenotypic character could be associated with S except for its effect on 19-80c. The S mutations of the *ps19-80* mutations could be mapped by crossing the irradiated revertants to various multiple-amber combinations which selected for the presence in the *am*⁺ progeny of the 19-80c mutation and another contiguous region of the genome of the revertant. For example, one might cross irradiated 19-80/BUR1 with *amE506/amE1270/amE355* and find that all of the *am*⁺ progeny are *ps19-80*(R1). (It should be kept in mind that sup-or will be rare among both the *am*⁺ progeny and the *am*⁻ progeny of this cross, because it enters the cross in the UV-irradiated parent, and no amber mutation is included which selects for its rescue.) This would mean that the S mutations of this revertant would be between *amE1270* and *amE355*. Should a subsequent cross, 19-80/BUR1(irr) × *amE506/amE1270/amE1236*, segregate a significant frequency of 19-80c among the *am*⁺ progeny, it would indicate that the S mutations do not lie between *amE1270* and *amE1236* and must therefore fall between *amE1236* and *amE355* (see the map locations of these mutations shown in Fig. 1B).

Five multiple-amber strains were crossed with each revertant which had been irradiated at three different UV doses. The surprising result of these crosses was that each *ps19-80* strain carried two S mutations. Specifically, these data showed that each revertant had within its genome two noncontiguous regions (defined by amber mutations in the helper phage), each of which was necessary but not sufficient to produce the *ps19-80* phenotype when combined with 19-80c. One region was the interval between N90 (gene 21) and *amE209* (gene 22), and the other interval was defined by *amE1236* (gene 23) and *amE355* (gene 24). The results of the crosses which demonstrate this observation for two revertants are shown in Table 6, along with control crosses with UV-irradiated *ptg19-80*. Five other revertants (19-80/BUR2, 19-80/

BURB2, 19-80/BURD1, 19-80/BURA1, and 19-80/BURA2) gave similar results. For each revertant, it was seen that rescue of the region N90-E355 (21 through 24) resulted in rescue of the *ps19-80* plaque type in virtually 100% of the *am*⁺ progeny. Rescue of the N90-E1236 region (21 through 23C) or the E209-E355 region (22 through 24) resulted in rescue of the *ps19-80* plaque type in a fraction of the *am*⁺ progeny. This fraction was inversely dose dependent, indicating that S or some component of S lay outside each of these regions. There was a new plaque type which was slightly smaller than the 19-80c plaque observed in some of these crosses. This plaque type evidently resulted from the combination of 19-80c with one of the components of some of the S mutations of the revertants and was designated <19-80c. The finding that a smaller plaque resulted when one of the suppressor mutations was combined with 19-80c suggested that this combination produced elevated levels of petite particles. These apparent double mutations were difficult to study because they gave rise to wild-type revertants at high frequencies, possibly owing to the large selective advantage that wild type would enjoy over a mutation that produced <4% plaque-forming particles (i.e., less than the percentage of plaque-formers produced by 19-80c).

Nomenclature. A new nomenclature will now be introduced to describe these suppressor mutations. The two new mutations of the *ps19-80* mutations will be termed x and y; i.e., S = x + y. The symbol x is assigned to the mutation in the N90-E209 region, with y being used to designate the mutation residing in the E1236-E355 region. The designation of a particular revertant is added after the x or y notation. Therefore, the genotype of revertant 19-80/BUR1 can now be written as sup-or/xR1/19-80c/yR1. Similarly, the genotype of *ps19-80*(R1) can be written as xR1/19-80c/yR1. The genotypes of relevant phage strains are described according to this nomenclature in Table 7.

Phenotypes of *ps19-80* mutations. One remaining question is: are the *ps19-80* strains revertants of 19-80c with respect to petite-phage production as well as plaque morphology? To answer this question, stocks of the *ps19-80* strains were prepared by method B, and the BKA ratios of these stocks were measured (Table 8). All of the mutations except *ps19-80*(F2LG) produced normal particles at a frequency very close to that of normal particles produced by *ptg19-80* (sup-or/19-80c). This result agreed well with the observation that the plaques produced by these *ps19-80* mutations were virtually indistinguishable from 19-80 plaques. In the same vein, *ps19-80*(F2LG) was clearly different in plaque morphology from 19-80, being larger (see Fig. 4).

TABLE 6. Mapping S mutations of *ps19-80* mutations

Cross of amber parent with revertant and UV dose (PLH) ^a	Frequency of various plaque types among <i>am</i> ⁺ progeny ^b					Frequency of <i>am</i> ⁺
	<19-80c	19-80c	<i>ps19-80</i>	Revertant	Wild type	
22/23A/23B/24 ^c × 19-80/BUR1						
24	0.14	0.04 (14)	0.82	0.09	0.01 (4)	1.22×10^{-3}
48	0.53	0.02 (5)	0.45			5.23×10^{-5}
96	0.57		0.43			1.93×10^{-6}
21/22/23A/23C ^d × 19-80/BUR1						
24	0.46	0.01 (4)	0.51	0.02	<0.01 (2)	1.30×10^{-3}
48	0.79	0.01 (8)	0.18		0.02 (10)	6.21×10^{-5}
96	0.89	0.02 (5)	0.08 (21)			2.88×10^{-6}
21/22/23A/23B/24 ^e × 19-80/BUR1						
24	0.05 (14)		0.93	0.02 (4)		7.22×10^{-4}
48			1.00			2.87×10^{-5}
96			1.00			3.20×10^{-7}
22/23A/23B/24 × 19-80/F2LG						
24		0.16	0.78	0.06	<0.01 (2)	9.51×10^{-4}
48		0.60	0.37	0.03 (6)		2.68×10^{-5}
96		0.66	0.34			7.49×10^{-7}
21/22/23A/23C × 19-80/F2LG						
24		0.53	0.47	0.01 (4)	<0.01 (2)	7.71×10^{-4}
48		0.76	0.21		0.03 (16)	5.23×10^{-5}
96		0.89	0.08 (13)		0.03 (4)	1.45×10^{-6}
21/22/23A/23B/24 × 19-80/F2LG						
24			0.99	<0.01 (1)	<0.01 (1)	6.90×10^{-4}
48			1.00			1.92×10^{-5}
96			1.00			5.70×10^{-7}
22/23A/23B/24 × <i>ptg19-80</i>						
24		0.97	0.03 (6)		<0.01 (1)	5.58×10^{-4}
48		0.99	<0.01 (1)			2.18×10^{-5}
96		1.00				4.47×10^{-7}
21/22/23A/23C × <i>ptg19-80</i>						
24		0.98	0.02 (7)		<0.01 (1)	7.52×10^{-4}
48		0.97			0.03 (11)	4.00×10^{-5}
96		0.96			0.04 (6)	1.38×10^{-6}
21/22/23A/23B/24 × <i>ptg19-80</i>						
24		0.99			0.01 (1)	4.82×10^{-4}
48		1.00				1.31×10^{-5}
96		1.00				1.84×10^{-7}

^a UV dose was as described in Table 5, footnote *b*.^b Revertant plaques arose from an infrequent rescue of sup-or; hence, they were more frequent at low doses. Wild type can occur by rescue of revertants (back mutations) at the 19-80c locus or by rare multiple recombinational events. <19-80c plaques are somewhat smaller than the 19-80c type.^c E209/E506/E1270/E355, see Fig. 1.^d N90/E209/E506/E1236, see Fig. 1.^e N90/E209/E506/E1270/E355, see Fig. 1.

The frequency of normal particles produced by *ps19-80*(F2LG) was about twice that of 19-80. The average phage yield per cell did not vary significantly among the mutants. These results provided further confirmation of the hypothesis that plaque morphology is a good reflection of the frequency of petite and normal particles produced.

The production of giant phage by *ps19-80* mutations was assayed by UV survival (Fig. 5). With the exception of *ps19-80*(F2LG), these mutations still produced significant amounts of giant particles. Like its parental revertant, 19-80/F2LG, the mutation *ps19-80*(F2LG) did not produce giant phage as assayed by UV survival. Electron microscopy examination of this stock

TABLE 7. Nomenclature of revertants

Symbols	Description
Single mutations	
19-80c	<i>ptg</i> mutation in gene 23
sup-or	Suppressor of 19-80c; located near lysozyme locus
x	Mutation(s) located between N90 (gene 21) and <i>amE209</i> (gene 22)
y	Mutation(s) located between <i>amE1236</i> (gene 23) and <i>amE355</i> (gene 24)
19-80	sup-or/19-80c
Revertants of 19-80	
19-80/BUR1	sup-or/xR1/19-80c/yR1
19-80/BUR2	sup-or/xR2/19-80c/yR2
19-80/F2LG	sup-or/xF2LG/19-80c/yF2LG
19-80/BURA1	sup-or/xA1/19-80c/yA1
19-80/BURA2	sup-or/xA2/19-80c/yA2
19-80/BURB2	sup-or/xB2/19-80c/yB2
19-80/BURD1	sup-or/xD1/19-80c/yD1
Pseudo-19-80 mutations	
<i>ps19-80</i> (R1)	xR1/19-80c/yR1
<i>ps19-80</i> (R2)	xR2/19-80c/yR2
<i>ps19-80</i> (F2LG)	xF2LG/19-80c/yF2LG
<i>ps19-80</i> (A1)	xA1/19-80c/yA1
<i>ps19-80</i> (A2)	xA2/19-80c/yA2
<i>ps19-80</i> (B2)	xB2/19-80c/yB2
<i>ps19-80</i> (D1)	xD1/19-80c/yD1
Other revertants	
19-80/BUR6	Not characterized
19-80/BURC1	Not characterized

of *ps19-80*(F2LG) also failed to detect the presence of any particles with longer-than-normal capsids.

Revertants 19-80/BUR6 and 19-80/BURC1. As previously noted, both revertant 19-80/BUR6 and revertant 19-80/BURC1 segregated high frequencies of 19-80c plaques when crossed with wild-type revertants. Crosses of these revertants to 19-80 and 19-80c indicated that they still retained sup-or. Attempts to map the additional suppressor(s) present in these revertants were unsuccessful. The results seemed to indicate (but did not prove) that there was no suppressor present that was tightly linked to gene 23. No further genetic analysis of these revertants was undertaken.

DISCUSSION

The mapping experiments described above suggested that x is located between N90 (gene 21) and *amE209* (gene 22), whereas y resides between *amE1236* (gene 23) and *amE355* (gene

TABLE 8. BKA ratios of *ps19-80* strains

Mutant	Ratio	
	BKA	1/BKA
<i>ps19-80</i> (R1)	8.6	0.12
<i>ps19-80</i> (R2)	10.3	0.10
<i>ps19-80</i> (A1)	9.9	0.10
<i>ps19-80</i> (A2)	8.9	0.11
<i>ps19-80</i> (B2)	7.3	0.14
<i>ps19-80</i> (D1)	8.0	0.13
<i>ps19-80</i> (F2LG)	4.3	0.23
<i>ptg19-80</i> ^a	8.3	0.12

^a Values from Table 2.

24). However, it is also possible that x is located to the left of N90 but is very tightly linked to it. This tight linkage could ensure that rescue of the plus allele of N90 always results in simultaneous rescue of x. Similarly, y could lie very near *amE355* but outside the region between *amE1236* and *amE355*. The linkages of x to N90 and y to *amE355* would need to be extremely tight, because a cross of *amE506* with 19-80(irr) at 96 PLH readily resolves the *am* and *ptg* sites. In such a cross, 30 to 40% of the *am*⁺ progeny failed to corescue 19-80c. Given the translational (5) and genetic maps of gene 23, one can esti-

FIG. 5. UV survival curves of *ps19-80* strains. Phage samples were irradiated and assayed for survival of PFU as described in the text. A wild-type (wt) control is shown in (A). The line generated by this control is drawn in all of the other panels (B through D). The wild-type data points are not shown, however. (A) Wild type (star) and *ps19-80*(R1) (●); (B) *ps19-80*(A1) (*) and *ps19-80*(R2) (●); (C) *ps19-80*(D1) (*) and *ps19-80*(B2) (●); (D) *ps19-80*(A2) (*) and *ps19-80*(F2LG) (●).

mate that *ame506* and *ptg19-80c* are separated by 100 nucleotide pairs. Thus, the most reasonable conclusion from the mapping experiments is that *x* is between N90 (gene 21) and *ame209* (gene 22). These genes are probably contiguous on the T4 genome (29). Of the two, gene 21 seems a far less likely location for the *x* mutation. The product of gene 21 (gp21) is a protease which performs all of the characterized cleavages associated with T4 capsid maturation (26). Conditionally lethal mutations in gene 21 (both amber and temperature sensitive) produce morphologically normal capsid precursors (pre-heads) when grown under restrictive conditions. Preheads made at a high temperature, owing to a temperature-sensitive mutation in gene 21, are not matured upon a temperature downshift (3). No length-determining role has ever been associated with gene 21. In contrast, gene 22 has been implicated in length determination by gene dosage experiments (27) and by the observation that at least some temperature-sensitive mutations in gene 22 produce high frequencies of petite phage when grown at semi-restrictive temperatures (24). Thus, the most probable location of *x* would seem to be in gene 22.

The *y* mutations are probably located between *ame1236* (gene 23) and *ame355* (gene 24). Data from UV rescue experiments suggested that it is closer to E355 than to E1236, which is our most C-terminal gene 23 amber mutation. E1236 is even more C terminal than *amC208*. The molecular weight of the gp23 polypeptide produced by C208 on *sup*⁰ bacteria is 80% that of the protein (5). Thus, *y* is more likely to be in gene 24 than in gene 23. Genes 23 and 24 have been found to be separated by about 500 base pairs of DNA which does not appear to code for any protein (M. Parker, personal communication). There are no other genes in this region that are known to be involved in morphogenesis of the capsid. Dosage of gene 24 has been shown to affect length determination (2, 7), as have certain temperature-sensitive mutations in gene 24 (2). From these considerations, gene 24 seems a reasonable candidate for the location of the *y* mutations.

The data presented here suggest that other T4-encoded proteins interact with gp23 in a manner that affects capsid-length determination. The other proteins implicated by these studies are gp22 and gp24 and possibly gpIPII or gpIPIII. However, it has not been demonstrated conclusively that the suppressor mutations do in fact reside in the genes encoding these proteins. The accompanying paper (10) extends these studies by providing further evidence for the location of suppressors in genes 22 and 24. More importantly, experiments are described which partially characterize the mechanism of suppressor ac-

tion and reflect upon the validity of current models of T4 capsid-length determination.

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