Genetic Studies on Capsid-Length Determination in Bacteriophage T4

II. Genetic Evidence that Specific Protein-Protein Interactions Are Involved

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Received 28 December 1981/Accepted 14 April 1982

A bacteriophage T4 mutation (*ptg19-80c*) located in gene 23, which encodes the major structural protein of the T4 capsid, results in the production of capsids of abnormal length. Mutations outside gene 23 which partially suppress *ptg19-80c* have been described in the accompanying paper (D. H. Doherty, J. Virol. 43:641-654, 1982). Characterization of these suppressors was extended. A complementation test suggested that the suppressors were in genes 22 and 24. These genes coded for the major component of the morphogenetic core of the capsid precursor and the vertex protein of the capsid, respectively. The suppressor mutations were found to have no obvious phenotype in the absence of *ptg19-80c*. Suppression was shown to be allele specific: other *ptg* mutations at different sites in gene 23 were not suppressed by the suppressors of *ptg19-80c*. These results indicated that specific interactions among the three proteins gp22, gp23, and gp24 may play a role in the regulation of T4 capsid-length determination. Current models for capsid-length determination are considered in the light of these results.

The accompanying paper (5) described the isolation and characterization of suppressor mutations of the bacteriophage T4 mutation *ptg19-80c*. This mutation causes the production of abnormal-length capsids. The suppressors significantly lowered the frequency of shorter-than-normal capsids. The suppressor mutations occurred in pairs in revertants of 19-80c. Mapping experiments indicated that one suppressor locus (x) resided in or near gene 22 and one locus (y) resided in or near gene 24. This paper extends the characterization of the revertants and uses them to study the mechanism of capsid-length determination.

The precise gene locations of the suppressors could not be determined by mapping. However, to properly interpret the suppression phenomena in light of models for capsid-length determination, it is necessary to know in which genes the suppressor mutations reside. A type of complementation test was therefore devised and carried out. The results of this test placed one suppressor locus (x) in gene 22 and the other (y) in gene 24. The phenotype of the suppressor mutations in the absence of *ptg19-80c* is also described. It is seen that these mutations ap-

pear to have no morphogenetic effects when *ptg19-80c* is absent.

The mechanism of suppression of *ptg19-80c* has been investigated by determining the range of specificity of these suppressors. Various multiple mutants were constructed which placed various *ptg* mutations in gene 23 in combination with the suppressors. Phenotypic characterization of these strains indicated that the suppressors were specific for mutations at the *ptg19-80c* site. This finding suggests that specific protein-protein interactions are involved in capsid-length determination.

MATERIALS AND METHODS

Bacteria. *Escherichia coli* strains B and CR63 were used exclusively in these studies. They are described in the accompanying paper (5).

Phage. The following amber strains of T4D from the collection of A. H. Doermann were employed: N90(21), *ame209*(22), *amb17*(23), *ame506*(23), *ame1270*(23), and *ame355*(24). The following *ptg* mutations, isolated by A. H. Doermann and located in gene 23, were used: *ptgBUA*, *ptg19-2*, *ptgH24*, *ptgS95*, *ptg19-80c*, *ptgNG212*, *ptgNG231*, *ptgA12N*, *ptgE65*, *ptgH21*, *ptgA24P*, *ptgA28R*, *ptgBB7*, *ptg191*, *ptgA20*, and *ptgH2*. The genetic map in Fig. 1 shows the relative locations of these mutations in gene 23.

All growth media and experimental techniques in the experiments reported here were employed as described in the accompanying paper (5).

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FIG. 1. Genetic map of gene 23 mutations. This map is from unpublished work of Doermann. Relative distances between amber markers are based on recombination frequencies. Amber (am) mutations are shown above the line, and petite and giant former (ptg) mutations are shown below this line.

RESULTS

Localization of x and y by complementation.

Mapping experiments described in the accompanying paper (5) indicated that seven revertants of *ptg19-80c* (termed *ps19-80s*) contained two suppressor mutations, one in or near gene 22 (x) and one in or near gene 24 (y). However, the mapping data did not prove the identity of the genes in which the suppressor mutations resided. To test the hypothesis that the suppressor mutations are located in genes 22 and 24, a complementation test was devised. Such a complementation test could, in principle, best be performed by making mixed infections on suppressor-minus (*sup*⁰) bacteria of various pseudo 19-80 (*ps19-80*) strains (x/19-80c/y) and the following series of single and multiple mutants: *ptg19-80c*, *am(22)/ptg19-80c*, *am(24)/19-80c*, and *am(22)/ptg19-80c/am(24)*. The simple prediction is that the mixed infection between *ps19-80* and 19-80c would result in reduced suppression (i.e., more petite-particle production) because the suppressor proteins of genes x and y are diluted by their wild-type counterparts which are brought into the infection by the 19-80c parent.

If x is in gene 22 and y is in gene 24, then the mixed infection between *ps19-80* and *am22/19-80c/am24* should result in restoration of the normal level of suppression, because only the suppressor forms of the proteins would be available to interact with the mutant capsid protein. The infections in which only one gene carries an amber mutation would be expected to yield increased frequencies of petite particles relative to *ps19-80* alone, since the genetic segregation pattern shows that both x and y are required for suppression.

Two factors complicated this experiment. One complication was that a series of attempts to construct multiple mutants involving *ptg19-80c* and amber mutations in genes 22 and 24 (also 23) failed. Therefore, an amber mutation in gene 23 was placed in all of the parents that carried the amber mutations; thus, mixed infections were always between a *ps19-80* strain and a phage that carried an amber mutation in gene 23 and one or more other genes. In this situation, the only functional gp23 produced was still a mutant (19-80c). The difference was that the amount of the protein was presumably reduced by 50%. This

TABLE 1. Complementation test

Mutation	BKA:1/BKA obtained with following amber parent ^a :				
	None	23 ⁻	22 ⁻ /23 ⁻	23 ⁻ /24 ⁻	22 ⁻ /23 ⁻ /24 ⁻
19-80c	30.2:0.033 28.0:0.036	17.2:0.058	29.1:0.034	19.0:0.053	25.0:0.040
<i>ps19-80</i> (A1)	9.9:0.10	10.1:0.10	16.3:0.06	9.0:0.11	5.8:0.17
<i>ps19-80</i> (A2)	8.9:0.11	10.1:0.10	14.8:0.07	8.3:0.12	4.1:0.24
<i>ps19-80</i> (B2)	7.3:0.14	8.0:0.13	13.2:0.08	7.8:0.13	5.4:0.19
<i>ps19-80</i> (D1)	8.0:0.13	9.9:0.10	15.4:0.06	9.0:0.11	3.9:0.26
<i>ps19-80</i> (R1)	8.6:0.12	7.2:0.14	14.0:0.07	8.2:0.12	4.1:0.24
<i>ps19-80</i> (R2)	10.3:0.10	9.6:0.10	13.9:0.07	10.0:0.10	5.9:0.17
<i>ps19-80</i> (F2LG)	4.3:0.23	4.5:0.22	8.2:0.12	4.2:0.25	2.1:0.48

^a Amber strains used were: 23⁻, *amE506/amE1270*; 22⁻/23⁻, *amE209/amE506/amE1270*; 23⁻/24⁻, *amE506/amE1270/amE355*; 22⁻/23⁻/24⁻, *amE209/amE506/amE1270/amE355*. The BKA ratio equals the ratio of total phage particles to plaque-forming particles and is measured as described in the accompanying paper (5). The reciprocal of the BKA ratio equals the fraction of plaque-forming particles present in the lysate.

raised the second complication, which was that differences in petite frequencies are known to arise from altered dosages of genes 22, 23, and 24 (1, 3, 6, 9, 10). The observation is that reducing the dosage of gp22 increases the frequency of the petite capsids, whereas reducing the dosage of gp23 or gp24 decreases the frequency of petite capsids. Therefore, control experiments must be performed by making mixed infections between the various amber strains employed and 19-80c to determine the effect of altered dosages of genes 22, 23, and 24 on petite-particle production by *ptg*19-80c. The results of these mixed infections must be the base line against which the results of the mixed infections involving *ps*19-80 strains should be measured.

The mixed infections made are shown in Table 1. The frequencies of petite phage particles from each mixed-infection lysate were measured as

described in the preceding paper. These experiments were performed with *E. coli* B (*sup*^h). The multiplicity of infection for each parent was 5. Some results with 19-80c were in good agreement with other observations on dosage effects; namely, reducing gp23 decreased the frequency of petite particles, and coordinately reducing gp22 and gp23 had no effect on petite-particle production. In contrast, there was little, if any, effect of limiting gp24. The mixed infection of 19-80c with 23⁻/24⁻ did not produce significantly fewer petite phage than the mixed infection of 19-80c with 23⁻. The same was true of 19-80c plus 22⁻/23⁻/24⁻ as compared with 19-80c plus 22⁻/23⁻. The results of the mixed infections of the *ps*19-80 mutations were similar to one another but different from the mixed infections involving 19-80c. The complementation experiment and the following interpretation of the results are summarized in schematic form in Table 2, using

TABLE 2. Summary of conclusions from complementation experiment^a

Parental phage	Gene			BKA:1/ BKA	Effects on petite-phage production		
	22	23	24		Dosage	Suppressor	Net
19-80c	WT	19-80c	WT	30.3:0.033	—	—	—
23 ⁻ 19-80c	WT WT	— 19-80c	WT WT	17.2:0.058	23 ↓, pt ↓	—	pt ↓
22 ⁻ /23 ⁻ 19-80c	— WT	— 19-80c	WT WT	29.1:0.034	22 ↓, pt ↑ 23 ↓, pt ↓	—	None
23 ⁻ /24 ⁻ 19-80c	WT WT	— 19-80c	— WT	19.0:0.053	23 ↓, pt ↓ 24 ↓, pt ↓	—	pt ↓
22 ⁻ /23 ⁻ /24 ⁻ 19-80c	— WT	— 19-80c	— WT	25.0:0.040	22 ↓, pt ↑ 23 ↓, pt ↓ 24 ↓, pt ↓	—	pt ↓ ? ~20%
<i>ps</i> 19-80(R1)	sup	19-80c	sup	8.6:0.12	—	—	—
23 ⁻ <i>ps</i> 19-80(R1)	WT sup	— 19-80c	WT sup	7.2:0.14	23 ↓, pt ↓	Mixing WT + sup proteins, pt ↑	None
22 ⁻ /23 ⁻ <i>ps</i> 19-80(R1)	— sup	— 19-80c	WT sup	14.0:0.07	22 ↓, pt ↑ 23 ↓, pt ↓	Mixing WT + sup proteins (gene 22), pt ↑	pt ↑
23 ⁻ /24 ⁻ <i>ps</i> 19-80(R1)	WT sup	— 19-80c	— sup	8.2:0.12	23 ↓, pt ↓ 24 ↓, pt ↓	Mixing WT + sup proteins (gene 24), pt ↑	None
22 ⁻ /23 ⁻ /24 ⁻ <i>ps</i> 19-80(R1)	— sup	— 19-80c	— sup	4.1:0.24	22 ↓, pt ↑ 23 ↓, pt ↓ 24 ↓, pt ↓	Only sup proteins present, no effect	pt ↓ 2×

^a Some results of the complementation test (from Table 1) are shown here, specifically, the BKA ratios produced by 19-80c and *ps*19-80(R1) (a typical representative of all of the *ps*19-80 strains) when each of these strains was grown on *sup*^h bacteria in the mixed infections with a series of amber mutations shown in the figure. The interpretation of the results is given in the column labeled effects on petite-phage production. This represents a summary of the arguments made in the text. Symbols: pt, frequency of petite-phage particles; ↓, decreases; ↑, increases; WT, wild type; sup, suppressor; 22⁻, *amE*209; 23⁻, *amE*506/*amE*1270; 24⁻, *amE*355; —, produces no protein.

data from *ptg19-80c* and only one pseudo 19-80 strain, *ps19-80(R1)*.

When *ps19-80* mutations mixedly infected with 23^- , no significant change in petite frequency (relative to *ps19-80* by itself) was observed. I attributed this to the action of two countervailing forces: the gene 23 dosage effect, which would tend to decrease petite frequencies, and the effect of wild-type *x* and *y* proteins (entering the infection with the 23^- parent), which would diminish suppression mediated by the mutant forms of *x* and *y* and thus increase petite frequencies. The observation that these two forces are about equal in magnitude (i.e., there is no change in petite frequencies) is presumably fortuitous. When *ps19-80* coinfects with $22^-/23^-$, increased frequencies of petite particles were observed. The $22^-/23^-$ combination had no dosage effect on 19-80c; therefore, this increase must have been due to inefficient suppression of 19-80c by mutant *x* and *y* proteins, presumably owing to the presence of wild-type *y* protein (gp24). The results of mixed infections of $23^-/24^-$ with *ps19-80* mutations were indistinguishable from those of mixed infections of *ps19-80* mutations with 23^- (as was the case with 19-80c). This suggests that the mixing of wild-type and mutant (suppressor) protein, in this instance presumably wild-type and mutant gp22, prevents efficient suppression and thus enhances the production of petite particles. This enhancement was counterbalanced by the dosage effect of limiting gp23 and possibly by a slight effect of limiting gp24. The behavior of the *ps19-80* mutations in mixed infections with $22^-/23^-/24^-$ was uniformly different from the behavior of 19-80c in the same mixed infection. The *ps19-80* mutations all showed significantly reduced frequencies of petite particles. These reductions were about 2-fold as compared with the *ps19-80* mutations alone and about 2.5- to 4-fold as compared with the mixed infection of $22^-/23^-$ plus *ps19-80*. The frequency of petite particles produced by the mixed infection of 19-80c plus $22^-/23^-/24^-$ did not significantly differ from the frequency of petite particles produced by either 19-80c alone or 19-80c plus $22^-/23^-$. This result is readily explained in a qualitative sense if *x* and *y* are in genes 22 and 24, because only mutant (i.e., suppressor) proteins are available to interact with gp23.

What is not explained is why this infection should produce fewer petite particles than *ps19-80* alone. A priori, one might have predicted that *ps19-80* plus $22^-/23^-/24^-$ would produce petite frequencies similar to those produced by *ps19-80* alone and *ps19-80* plus 23^- , whereas 19-80c plus $22^-/23^-/24^-$ would give a frequency of petite particles like those of 19-80c alone but greater than those of 19-80c plus 23^- . Perhaps simulta-

neous variation of the dosages of genes 22, 23, and 24 has some effect on the suppressor proteins that is not observed with the wild-type alleles. In any event, the results of these experiments were consistent with the locations of *x* and *y* being those suggested by the mapping experiments and with the known effects on length determination of genes 22 and 24.

Phenotypes of *x* and *y* mutations. The phenotypes of the suppressor mutations *x* and *y* were investigated. These mutations appeared to have no phenotypes except for their effects on the 19-80c mutation. Simple genetic crosses of the revertants of 19-80c by *amB17* (gene 23) and *amE209* (gene 22) yielded the same frequencies of wild-type recombinants as crosses of these two amber mutations by the parent *ptg19-80* (*sup-or/19-80c*). Because *x* is located near *amE209*, almost all *am⁺/19-80c⁺* progeny of these crosses will carry *x*. These wild type were indistinguishable from the control. Analogous results regarding the *y* mutation were obtained with crosses of the revertants and *ptg19-80* by *amE1236* (gene 23) and *amE355* (gene 24). Some combinations of *x/19-80c* and *19-80c/y* appeared to alter the plaque morphology of 19-80c. This was observed in the mapping experiments described in the preceding paper. The individual effect, if any, of *x* or *y* on 19-80c was always to reduce the plaque size. There was no evidence that any *x* or *y* mutation could act alone to suppress 19-80c.

The phenotypes of two combinations of *x*, *y*, and *sup-or* (an additional suppressor of 19-80c described in the accompanying paper [5]) were examined more closely. The 19-80c mutation was removed from two revertants of *ptg19-80* (19-80/BUR1 and 19-80/BUR1A1) to produce strains of the following genotypes: *sup-or/xR1/yR1* and *sup-or/xA1/yA1*. Stocks of these mutants produced by method B as described in the accompanying paper (5) had bacteria killing ability (BKA) ratios of 0.94 and 1.05, respectively. No giant phage particles were detected by kinetics of UV inactivation or electron microscopy examination of the stock. The plaque morphology of *sup-or/xR1/yR1* (BUR1) was indistinguishable from that of the wild type. Strain BUR1A1 (*sup-or/xA1/yA1*) produced a slightly smaller plaque than did a wild-type control.

The isolation of these strains and the verification of their genotypes were accomplished as follows. The revertants were crossed by UV-irradiated *amE506*, a gene 23 mutation closely linked to 19-80c. From these crosses, the infrequent amber progeny were identified by testing numerous individual progeny plaques for growth on *sup⁰* and *sup⁺* hosts. This is, in effect, a "rescue" of the *amE506* amber allele into the genetic background of the revertant. The aim is

to replace the mutation 19-80c locus with its wild-type allele, which ought to be nearly always coresced with the amber allele of the *amE506* locus. If this was accomplished without simultaneous corescue of either x^+ or y^+ (or *sup-or*⁺), then the resulting *amE506* progeny had the genotype *sup-or/x/amE506/y*. These strains were designated *amE506/BUR* strains, specifically, *amE506/BUR1* and *amE506/BUR1*. The identity of these strains was verified by crossing them to heavily irradiated *ptg19-80* or *ptg19-80c*. The *am*⁺ progeny of these crosses ought to have been, and were, revertant, as opposed to 19-80c, if the surmised genotypes were correct. From the *amE506/BUR* strains, the *am*⁺ *sup-or/x/y* strains were readily derived by selection of spontaneous amber-plus revertants of *amE506/BUR* or by crossing *amE506/BUR* by heavily UV-irradiated wild type.

Specificity of suppression. The questions that now arise are: how do the suppressor mutations act on the 19-80c locus, and what does this reveal about capsid-length determination and the various models proposed to describe length determination of the T4 capsid? The current models for T4 capsid-length determination would seem to make rather different predictions about the specificity of the suppressor mutations. Seemingly, the most direct prediction of the kinetic model (10) would be that the suppressors act by altering the effective intracellular protein concentration of gp22, gp24, and gp(*sup-or*), possibly gpIPII or gpIPIII. The amounts of the core proteins gp22 and gp(*sup-or*) [if gp(*sup-or*) is a core protein] should be increased, and the intracellular concentration of gp24 should be reduced. By the kinetic model, this would result in production of a decreased fraction of petite particles. It seems implicit that, under the kinetic model, *ptg* mutations at other sites in gene 23 should also be suppressed by the suppressor mutations, because a priori they also ought to be sensitive to dosage effects. Models of length determination which are based on specific protein-protein interactions, such as the vernier model (9) and the cumulated-strain model (7), would accommodate rather different predictions. These models would suggest that the mutations in the suppressors affect active sites on the various proteins and that these sites influence interactions (direct or indirect) between gp23 and these proteins. Such interactions would exert an influence on length determination. Specific models would readily accommodate observations of specificity in the range of *ptg* mutations suppressed by any given suppressor. That is, some *ptg* mutations might be suppressed, others not, and this property might be related to the genetic map location of the *ptg* mutation.

To determine the range of suppressor specificity, it was necessary to construct new multiple mutants which combined the suppressor mutations of a given revertant (*sup-or/x/y*) with a *ptg* mutation other than 19-80c. There are two serious obstacles to the construction of such multiple mutants. First, *x* and *y* are closely linked to, and flank, gene 23, wherein the *ptg* mutations reside. This necessitates a double-crossover event within a relatively short interval to produce the desired recombinant; in any cross between a *ptg* and a phage carrying *x* and *y*, the progeny of interest will be quite rare. A second, more troublesome problem is that one cannot know a priori whether the recombinant genotype (*sup-or/x/ptgZ/y*) will be distinguishable from that of the *ptgZ* parent. For example, one might cross *ptgZ* with BUR1 (*sup-or/xR1/yR1*) to isolate *ptgZ/BUR1* (*sup-or/xR1/ptgZ/yR1*). If *ptgZ* is not influenced by these particular suppressor mutations, it might well be that the plaque morphologies of *ptgZ* and *ptgZ/BUR1* would be identical. Slight differences in plaque morphology are also difficult to observe if one type is in substantial excess.

These problems can be overcome by employing a UV rescue experiment. In this case, one can take advantage of the previously isolated *amE506/BUR* strains. It should be recalled that these strains carry all of the mutations necessary to convert 19-80c into a particular revertant, and the *amE506* mutation in gene 23 (which is closely linked to the 19-80c locus), but lack the 19-80c mutation. If an *amE506/BUR* strain is crossed with a heavily irradiated *ptg* mutant [e.g., *sup-or/xR1/amE506/yR1* × *ptgZ(irr)*], then most *am*⁺ progeny will corescue the *ptgZ* mutation along with the plus allele of *amE506* and will therefore have the genotype *sup-or/xR1/ptgZ/yR1*. This is essentially the same experiment described previously that was used to prove the identity of the putative *amE506/BUR* strains. The strength of this test is that we know [from the cross *amE506/BUR1* × *ptg19-80(irr)*] that the predominant genotype among the amber-plus progeny must be *sup-or/xR1/ptgZ/yR1*, although there are also some *sup-or/xR1/yR1* progeny (i.e., BUR1) present which result from rescue of the plus allele of the amber mutation without corescue of the *ptgZ* mutation. Therefore, one can use plaque morphology as a rapid and convenient measure of suppression of various *ptg* mutations by given BURs.

The experiment that was done was simply to cross two *amE506/BUR* strains with heavily irradiated *ptg* mutants. The UV doses used were chosen to give significant proportions of wild-type-appearing *am*⁺ progeny (20 to 65%). The frequent presence of wild-type-appearing progeny (genotype *sup-or/x/y*) among the *am*⁺ proge-

ny indicated that a significant proportion of the recombinational events which rescue the plus allele of *amE506* failed to rescue the *ptg* mutation; the corescue of the more distant x^+ and y^+ alleles must occur far less frequently. Hence, it could be concluded that, among the progeny of each cross, there is a significant population of am^+ progeny having the genotype *sup-or/x/ptgZ/y*. If a cross such as *amE506/BUR1* with *ptgZ* results in only wild-type and *ptgZ* plaques when plated on *sup*⁰ bacteria, it can be inferred that *ptgZ* is not suppressed by the BUR1 suppressor mutations.

Sixteen *ptg* mutations (map location shown in Fig. 1) were each irradiated and crossed with *amE506/BUR1* and *amE506/BURA1*. Progeny of these crosses were plated on *E. coli* B and incubated at 37°C for 48 h, at which time they were scored for plaque morphology. The morphologies of the progeny plaques from these crosses were compared with those of controls, which consisted of similar *sup*⁰ platings of progeny from crosses of the irradiated *ptg* mutations with *amE506* (Table 3). Nearly all *ptg* mutations tested showed no effects from being combined with the various suppressors. The exceptions included 19-80c and three other *ptg* mutations located at the same site as 19-80c. These mutations were all dramatically suppressed, as determined by plaque morphology. Two other mutations, located at a different site in the same cluster as the 19-80c site (see map of *ptg* sites in Fig. 1), appeared to be enhanced (i.e., made smaller, the opposite of suppressed) by the suppressor mutations. These results indicated that suppression is allele specific. Such specificity is not consistent with a model in which suppressors act by altering the intracellular amounts of proteins. Allele specificity is consistent with the notion that suppressors do affect protein-protein interactions involved in capsid-length determination.

The results concerning suppression or the lack of it are all dependent on the proposition that plaque morphology is a good reflection of the frequency of petite particles produced. The results of previously described experiments in the accompanying paper (5) have provided strong support for this idea. As further verification, BKA ratios of stocks made from a number of the segregants described in Table 3 were measured. The multiple-mutation strains studied included instances of suppression and enhancement as well as cases in which no effect of suppressor mutations was observed. The multiple mutants used and their BKAs are listed in Table 4. These results again provide good confirmation of the correlation between plaque morphology and production of petite particles. Segregants classified as suppressed by the criterion of plaque

morphology produced fewer petite phage than did the parental *ptg* from which they were derived. The converse was true for those strains which were classified as enhanced by virtue of producing a smaller plaque. Those strains which produced plaques indistinguishable from those of their respective *ptg* parents also produced petite particles at approximately the same frequency as that of their parents. The conclusions based on plaque morphology thus seem well substantiated.

DISCUSSION

Until rather recently, theories for the mechanism of length determination of the T4 capsid have been predicated on the assumption that specific protein-protein interactions are central

TABLE 3. Suppressor specificity: resulting effect on plaque morphology of crosses of *ptg(irr)* by *E506/BUR*

<i>ptg</i> mutation	Result of cross with following amber parent ^a :	
	<i>amE506/BUR1</i>	<i>amE506/BURA1</i>
H2	NC	NC
A20	NC?<	NC
191	NC	NC
BB7	NC	NC
A28R	E	E
A24P	E	E
H21	NC	NC
E65	NC	NC
A12N	S	S
NG231	S	S
NG212	S	S
19-80c	S	S
S95	NC	NC
H24	NC	NC
19-2	NC	NC
BUA	NC	NC

^a All crosses of *ptg(irr)* by *amE506/BURA1* produced wild type which were slightly smaller than control wild type. In addition, *ptg/BUR* progeny were seemingly reduced proportionally in size as compared with the *ptg* control. This appears to be an effect solely on plaque morphology. NC, No change; i.e., plaque morphologies of am^+ progeny are identical to those of controls. ND?<, Plaque morphologies of am^+/ptg progeny were very slightly smaller than those of control. E, Enhanced; i.e., plaque morphologies of am^+/ptg progeny were smaller than those of *ptg* control. S, Suppressed, i.e., plaque morphologies of am^+/ptg progeny were larger than those of *ptg* control.

TABLE 4. Suppressor specificity: correlation of plaque morphology and petite-phage production

Mutation	Suppressor effect ^a	BKA	1/BKA
19-2		4.0	0.25
19-2/BURA1	NC	3.6	0.28
A12N		25.0	0.04
A12N/BUR1	S	3.6	0.28
A20		5.1	0.20
A20/BUR1	NC?<	5.5	0.18
A28R		3.3	0.30
A28R/BUR1	E	6.9	0.14
A24P		3.6	0.28
A24P/BUR1	E	6.6	0.15

^a NC, No change in plaque morphology; S, suppressed, i.e., larger plaques than *ptg* parental control; E, enhanced, i.e., smaller plaques than *ptg* parental control.

to the length determination process. Models of capsid-length determination have tacitly assumed that length determination is independent of capsid subunit concentrations. However, Showe and Onorato (10) have proposed a model for T4 capsid-shape determination in which the length of the capsid is determined by the relative rates of assembly of the morphogenetic core and the capsid shell. This implies that the process of length determination is controlled by the intracellular concentrations of the protein precursors of these structures. The model of Showe and Onorato, which the authors term a kinetic model, proposes that the morphogenetic core and the gp23 shell of the prehead elongate independently. The lengths of the prehead and the mature capsid are determined by the relative rates of growth of these two structures and the rate of vertex formation or stabilization or both. The authors suggest that the assembling gp23 shell has an intrinsic tendency to curve in both the axial and equatorial directions of the nascent prehead. The intrinsic axial curvature of the shell favors formation of vertices which are closer together longitudinally than they are actually observed to occur normally. This tendency for premature termination of elongation is counteracted by the morphogenetic core. The core sterically inhibits the shell from achieving its greatest potential curvature.

By the kinetic model, gene 23 mutations affecting capsid length would do so either by affecting the intrinsic curvature of the assembled gp23 shell or by decreasing the interaction of the shell with the core. This could be equivalent to lowering the effective concentration of gp22, which is the principal essential protein of the

core. These two mechanisms were previously proposed by Paulson and Laemmli (8) to explain the effects of mutation E920g. There is evidence that the intrinsic curvature of assemblies of gp23 is affected by core proteins. Amber mutations of gene 22 produce aberrant assemblies of gp23. These assemblies, called polyheads, are long, tubular structures which do not contain cores. It has been reported that 22⁻ polyheads have a smaller average diameter than polyheads containing cores (11). Mutation *ptg*E920g might act by increasing the intrinsic curvature of assembled gp23 in the axial direction. This could lead to the production of petite particles.

Direct support for the kinetic model comes from experiments on the effects of gene dosage on capsid-length determination. In these experiments, mixed infections are made between wild type and a phage carrying an amber mutation in the gene of interest. The infected host is suppressor minus (*sup*⁰), and thus the gene dosage and, presumably, the intracellular amount of the protein of interest can be altered by adjusting the relative input ratio of the parental phage. The results of these experiments indicated that reduction of gp23 results in reduction of the frequency of petite phage particles produced (6, 10), whereas reduction of gp22 increases the frequency of petite capsids (1, 10). Decreased levels of gp24 have been found to decrease the frequency of petite particles (10) but to result in production of some particles with giant capsids (2, 3). Results consistent with these findings have also come from experiments involving growth of temperature-sensitive mutants at a near-restrictive temperature. This might be equivalent to reducing the effective concentration of mutant protein. The observations are that temperature-sensitive mutations in gene 22 result in production of high frequencies of petite particles at near-restrictive temperatures (9), and temperature-sensitive mutations of gene 24 cause some giant phage particles to be produced at high temperatures (2).

The kinetic model postulates that both core and shell influence length determination specifically through their relative rates of assembly. Previous models which have been predicated on specific protein-protein interactions have implicated interactions between protomers of the gp23 shell, as in the cumulated-strain model of Kellenberger (7), or interactions between the shell and the morphogenetic core, as in the vernier model of Paulson and Laemmli (8), as the mechanism of length determination. An alternative proposal has been made by van Driel and Couture (13). They have put forth a model which states that, in vivo, the morphogenetic core is assembled first. After completion of the core, a shell of gp23 is assembled around this

"naked" core. This model is based on in vitro studies of prehead assembly (12, 13). It was observed that core proteins extracted from preheads assembled in vivo can form corelike structures of roughly appropriate dimensions in vitro when gp23 is not present. The authors concluded that all of the architectural information for capsid-shape determination resides in the core proteins and is expressed during the assembly of naked cores. This model is in apparent contradiction to a number of previous results, including the known effects of gene dosage on capsid-length determination (10), particularly the effect of gene 23 dosage, and the isolation of numerous point mutations in gene 23 which affect the fidelity of capsid-length determination (4; Doermann, unpublished results). It does not appear to be possible to incorporate these facts into a model of prehead assembly in which gp23 polymerizes around a prefabricated naked core of already-determined length. Possibly, the in vitro formation of such a structure is due to the absence of gp23 from the reaction. It is known that, in the absence of the major essential core component, gp22, the major essential capsid protein, gp23, forms long, hollow tubes both in vitro (12) and in vivo (11). These structures, termed polyheads, clearly do not represent the normal mode of assembly of gp23 in vivo when all of the normal components of capsid morphogenesis are available. From these considerations, it is argued that the formation of naked cores has yet to be demonstrated as part of the normal in vivo prehead assembly pathway.

In the studies described here and in the accompanying paper (5), it has been demonstrated that the morphogenetic phenotype of a gene 23 mutation, *ptg19-80c*, is partially suppressed by a mutation, designated sup-or, which was shown to map near the T4 gene for lysozyme. This suppressor may reside in one of two loci in that region which code for internal proteins, IPII and IPIII, that are nonessential components of the morphogenetic core. However, no direct evidence demonstrates the genic identity of sup-or. Before the discovery of the true genetic constitution of *ptg19-80* (19-80c/sup-or), nine pseudo-wild-type revertants of 19-80 were isolated. These revertants all produced significantly reduced frequencies of petite particles as compared with 19-80c and 19-80, but all still produced some petite particles. Seven of these revertants carried two new suppressor mutations, one located in or near gene 22 and one in or near gene 24. Both of these mutations were simultaneously required for suppression. In the absence of sup-or, these two mutations still suppressed 19-80c, but the efficiency of suppression was reduced. None of the suppressor mutations, alone and in various combinations, were

found to have any discernible phenotype in the absence of *ptg19-80c*.

The nature of the mechanism by which these suppressors act on the 19-80c mutation was investigated. Multiple mutants were constructed which combined sup-or and the suppressor mutations, x and y, of a revertant with various other *ptg* mutations in gene 23. In most instances, no effect of the combined suppressors on the *ptg* phenotype could be detected. Among the exceptions were three *ptg* mutations which occurred at the same site as 19-80c, i.e., they failed to recombine with 19-80c. These mutations were all efficiently suppressed by both suppressor genotypes (sup-or/xR1/yR1 and sup-or/xA1/yA1) tested. The only other exceptions were two mutations which both occurred at a single site different from the 19-80c site. These mutations were enhanced by the two suppressor genotypes tested. By enhanced, it is meant that these suppressors caused the *ptg* mutations to produce higher frequencies of petite particles.

On the basis of these results, the following conclusions were reached.

(i) The allele specificity displayed by the suppressor mutations suggests that there are specific protein-protein interactions which can influence length determination. In particular, the observation that the same suppressor can suppress some mutations, enhance others, and not affect still other mutations argues that an alteration of some specific protein-protein interaction(s) is the basis of suppression, rather than an alteration in the effective intracellular concentration of gene products.

(ii) Genetic evidence indicates that suppressors can occur as pairs of mutations, one member of each pair mapping in or near gene 22 and one member mapping in or near gene 24. Data from complementation experiments are also consistent with the interpretation that the suppressors reside in genes 22 and 24. Both mutations are simultaneously required for suppression, suggesting that gp23 may interact with both gp22 and gp24 in the process of length determination. Knowledge of the roles of these proteins in capsid morphogenesis indicates that the opportunity for such interactions exists. For example, it could be that the proteins of the nascent capsid shell (gp23) and the nascent core (gp22) form a complex with which the vertex protein (gp24) interacts. This putative interaction could be involved in termination of axial elongation of the nascent prehead.

(iii) Finally, these results suggest that T4 capsid-length control may be simultaneously subject to effects of specific interactions of the core, shell, and vertex proteins as well as kinetic effects of variations in the intracellular concentrations of these proteins.

ACKNOWLEDGMENTS

I thank Gus Doermann for continual interest and advice during the course of these studies.

This research was supported by Public Health Service grant GM 13280 and by Public Health Service Training grant GM 00812 from the National Institute of General Medical Sciences.

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