JOURNAL OF VIROLOGY, Dec. 1975, p. 1547-1559
Vol. 16, No. 6

Printed in U.S.A.

Bacteriophage P22 Virion Protein Which Performs an Essential Early Function

II. Characterization of the Gene *16* Function

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Received for publication 1 August 1975

P*16* is a virion protein and, as such, is incorporated into the phage head as a step in morphogenesis. The role of P*16* in assembly is not essential since particles are formed without this protein which appear normal by electron microscopy. P*16* is essential when the particle infects a cell in the following cycle of infection. In the absence of functional P*16*, the infection does not appear to proceed beyond release of phage DNA from the capsid. No known genes are expressed, no DNA is transcribed, and the host cell survives the infection, continuing to grow and divide normally. The P*16* function is required only during infection for the expression of phage functions. Induction in the absence of P*16* proceeds with the expression of early and late genes and results in particle formation. P*16* must be incorporated during morphogenesis into progeny particles after both infection and induction for the progeny to be infectious. The P*16* function is necessary for transduction as well as for infection. Its activity is independent of new protein synthesis and it is not under immunity control. P*16* can act in *trans*, but appears to act preferentially on the phage or phage DNA with which it is packaged. The data from complementation studies are compatible with P*16* release from the capsid with the phage DNA. In the absence of P*16* the infection is blocked, but the phage genome is not degraded. The various roles which have been ruled out for P*16* are: (i) an early regulatory function, (ii) an enzymatic activity necessary for phage production, (iii) protection of phage DNA from host degradation enzymes, (iv) any generalized alteration of the host cell, (v) binding parental DNA to the replication complex, and (vi) any direct involvement in the replication of P22 DNA. P*16* can be responsible for: (i) complete release of the DNA and disengagement from the capsid, (ii) bringing the released DNA to some necessary cell site or compartment such as the cytoplasm, (iii) removal of other virion proteins from the injected DNA, and (iv) alterations of the structure of the injected DNA.

As reported in the accompanying paper (13), and by Botstein et al. (9) and King et al. (16), P*16* is a head protein of the *Salmonella typhimurium* temperate bacteriophage P22. This protein plays no role in phage morphogenesis, but is necessary for the particle to be infectious in the next cycle of infection. In the accompanying paper (13), we describe a temperature-sensitive gene *16* mutant which has an early phenotype. No phage DNA synthesis takes place at the restrictive temperature (6). Temperature shift experiments with P22 *16⁻ts* clearly show that on infection P*16* is needed very early to initiate the sequence of infective events. However, P*16* is not required on induction for the expression of vegetative phage genes. Viable phage production after either infection or induction requires that P*16* be incorporated into progeny particles during morphogenesis (13).

Defective particles missing P*16* are produced (i) during a nonpermissive P22 *16⁻am* infection, (ii) when a P22 *16⁻ts* infection initiated at 25 C is shifted to 41 C early in the infection, and (iii) on induction of a *16⁻am* or *16⁻ts* mutant lysogen under nonpermissive conditions (9, 13). P22 *16⁻ts* phage, grown under permissive conditions, appear to possess a thermolabile P*16* which, although heat stable in the virion, is inactivated on infection at 41 C (13). Thus abortive infection occurs, due to the absence of functional P*16* when P22 *16⁻ts* is used at 41 C, or when defective particles are used to infect any host at any temperature.

Little is known about the early stages of infection, between adsorption of the virion to

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the host cell and the association of parental phage DNA with the replication complex (22). These gene *16* mutant phage and defective particles offer a means to study the early gene *16* function, thereby gaining insights into a very early phage activity. The experiments presented in this paper attempt to elucidate the early phage function carried out by P16. Although we can still only speculate about the nature of this function, many alternatives are eliminated. Possible roles for P16 are discussed.

MATERIALS AND METHODS

Bacteriophage strains, bacterial strains, media, and methods not described here are presented in the accompanying paper (13).

Bacteriophage strains. P22 *16⁻ts16.1* was isolated in this laboratory from wild-type phage P22 mutagenized with 1-methyl-3-nitro-1-nitrosoguanidine. R. N. Rao isolated P22 *sie1* in this laboratory. Prophage bearing *sie* mutations do not exclude superinfecting phage but are still immune to phage P22 (27, 34). Phage L was obtained from Amati.

With the exception of temperature-sensitive mutations in gene *16*, only one mutant allele from each gene was used in this report. Phage carrying these mutations are referred to by gene number and the type of conditional lethal, *ts* or *am*, if applicable. For *16⁻ts* mutants, the particular allele *ts16.1* or *ts16.2* is given.

Bacterial strains. A galactose-negative mutant of *S. typhimurium* LT2 strain 18 was used as the indicator on eosin methylene blue (EMB) galactose agar plates and is designated strain 2. Lysogens 192 (*L*) and 18 (*c⁺sie*) were isolated after infection at 37 C. Lysogenic strain 18 (*12⁻amc2⁻tssie*) was isolated after mixed infection with the clear, nonlysogenizing P22 *c2*. Recipients for transduction experiments were strain LT2 carrying the *pro C90* mutation (30) and strain LT2 carrying the *hisG203* mutation (obtained from P. E. Hartman). These strains were lysogenized with P22 *sie* prophage so that they are immune to superinfecting phage. Thus, none of the cells are killed by infection while transductants are being selected.

Media. L broth (21), M9CAA medium (30), EMB galactose agar (21), and minimal agar (29) have been previously described.

Preparation of [³H]thymidine labeled phage. Log-phase cells, grown in M9CAA medium to a concentration of 2×10^8 cells/ml, were infected with P22 phage (multiplicity of infection [MOI] of 10). At the time of infection, deoxyadenosine, at a final concentration of 250 μg/ml, and [³H]thymidine (specific activity = 22.3 Ci/mmol), at a final concentration of 10 μCi/ml, were added to the cell suspension. After lysis, the phage were concentrated and purified in CsCl as described (13).

Preparation of defective particles missing P16. These particles were produced in any of the following ways: (i) P22 *16⁻am* nonpermissive infections; (ii) P22 *16⁻ts* infection initiated at 25 C and then shifted to 41 C after 10 min; or (iii) induction of *16⁻ts* or *16⁻am* lysogens under restrictive conditions. After lysis, the particle suspension was concentrated and purified as described in the accompanying paper (13).

In this and the accompanying paper, the term defective particles will refer to noninfectious particles produced after either infection or induction with gene *16* mutant phage under nonpermissive conditions. When the genotype of these particles is given, it will precede in this term. The manner by which the particles were produced, either infection or induction, will follow in parentheses. Thus, defective particles formed by induction of *16⁻ts* lysogens at the nonpermissive temperature will be designated *16⁻ts* defective particles (induction).

Starving cells for glucose. Cells were grown in supplemented M9CAA to a concentration of 2×10^8, washed twice with M9CAA without glucose, resuspended in M9CAA without glucose, and aerated for 90 min.

Assay for acid solubilization of phage DNA. The assay measures the in vivo conversion of [³H]thymidine-labeled parental phage DNA to acid-soluble nucleotides and oligo-nucleotides after infection. One-half milliliter of the infected cell suspension was added to 0.2 ml of cold calf thymus DNA (2.5 mg/ml), and the in vivo reaction was stopped by addition of 0.3 ml of cold 7% perchloric acid. After centrifugation at 10,000 rpm for 10 min in a Sorvall SM-24 rotor, the supernatant was decanted. One-half milliliter of this supernatant was added to 10 ml of *p*-dioxane scintillation medium (10) and counted in a Packard Tri-Carb scintillation spectrometer.

Detection of cells carrying phage genomes. Cells to be tested were plated by the soft agar-overlay method with a background of galactose-negative cells (strain 2) on EMB-galactose agar plates. Plates were incubated for 48 to 60 h at 25 C. Cells carrying phage genomes gave rise to colonies with a surrounding halo of lysis due to spontaneous phage release, whereas cells not carrying phage genomes did not (21).

Segregation experiments. Log-phase cells were infected at the designated temperature with a multiplicity of 20 phage and diluted after a 5-min adsorption period to 10^3 cells/ml in M9CAA medium containing anti-P22 serum (K = 4). At intervals, samples were plated on EMB-galactose agar as described above for determination of numbers of sensitive and phage-carrying cells.

Transduction. Particles from thermally induced 18 (*c2ts*) and 18 (*16⁻ts16.2c2ts*) lysogens and particles from P22 *c1*- and P22 *16⁻amc1*-infected *su*-strain 18 were concentrated and purified in CsCl. The absolute number of particles in each purified phage suspension was estimated from the optical density of the suspension at 260 nm (29). The auxotrophic recipient cells were made lysogenic with P22 *sie*, thus eliminating complications produced by killing. The recipient cells were grown to a concentration of 3×10^8 to 5×10^8 cells/ml in L broth, washed twice with buffered saline, and infected with the appropriate phage suspension (MOI = 5 to 10). These infected cells were diluted and plated on minimal agar plates to assay for prototrophic transductants.

RESULTS

Infection with phage missing functional P16. Botstein et al. (9) and King et al. (16) report that P22 particles missing P16 are not viable. Levine et al. (23) have shown that, 4 min after the initiation of infection, parental phage DNA rapidly accumulates in the replication complex (intermediate I), reaching a peak at about 15 min. However, P22 *16⁻ts* parental DNA does not associate with this complex at high temperature. In a nonpermissive P22 *16⁻ts* infection, no phage DNA or protein synthesis is detected (6). To help characterize the P16 function a number of manifestations of the expression of early phage activities were monitored on infection with either defective particles or P22 *16⁻ts* at nonpermissive temperatures. Among these parameters are host survival, phage adsorption, release of viral DNA from the capsid, expression of exclusion of superinfecting phage, lysogenization of the host, and titration of repressor.

Host survival. To ascertain whether the host cell survives infection with *16⁻amc1*-defective particles (infection), the colony-forming ability of the infected cells was examined. There is no difference in the number of colonies formed by cells exposed to these defective particles compared to the unexposed control cells. It has also been shown that cells exposed to P22 *16⁻ts16.2c2* at 41 C continue to grow and divide with the same kinetics as uninfected cells (B. Hoffman, Ph.D. thesis, Univ. of Michigan, Ann Arbor, 1973). These data are in agreement with the observations of Bezdek and Soska (6).

Adsorption. Log-phase cells, at a concentration of 10^8 cells/ml, were exposed to P22 *c2* (MOI = 14), P22 *16⁻ts16.2c2* (MOI = 10), P22 *c2* (MOI = 0.6), and *16⁻ts16.2c2* defective particles (shifted-up infection; MOI = 0.8) at 41 C. At various times, samples were diluted into buffered saline plus a few drops of chloroform, immediately shaken to destroy the cells, and assayed for either free phage or defective particles. Unadsorbed defective-particle titers were determined using the infective center assay (13). The adsorption velocity constants (k) were calculated for each infection (1). The average k values obtained are: 1.7×10^{-8} for P22 *c2* (MOI = 14); 2.1×10^{-8} for P22 *c2* (MOI = 0.6); 2.3×10^{-8} for P22 *16⁻ts16.2c2*; and 2.2×10^{-8} for *16⁻ts16.2c2* defective particles (shifted-up infection). Thus, phage with nonfunctional P16 adsorb to host cells with normal kinetics.

Release of DNA. When cells starved for glucose are infected, a large fraction of the parental phage DNA becomes acid soluble. This breakdown of DNA does not occur during infection of actively growing cells. The mechanism of this phenomenon is not understood, but it is presumed to be due to the accessibility of the phage DNA to host nucleases in the starved cells. These observations offer a method to determine whether the DNA from particles without functional P16 is released from the capsid. The finding of acid-soluble parental phage DNA after adsorption to starved cells will be taken as a measure of the release of the DNA from the virion capsid.

Cells grown to a concentration of 2×10^8/ml were suspended in M9CAA without glucose and aerated for 90 min at 37 C. The cells were then warmed to 41 C and infected with [^{3}H]thymidine-labeled P22 *c2*-, P22 *16⁻ts16.2c2*-, or *16⁻am*-defective particles (infection). At various times after infection, samples were assayed for acid-soluble DNA.

The amount of parental phage DNA which has become acid soluble is extensive for all three infections (Table 1). By 62 to 64 min, 61% of the *c2* DNA, 64% of the *16⁻tsc2* DNA, and 67% of the *16⁻am*-defective particle DNA are broken down to acid-soluble products. Thus, on infection of starved cells, the parental DNA from phage missing functional P16 is rendered acid soluble to the same extent as P22 *c2* DNA. We conclude that, in the absence of functional P16, phage DNA is released from the capsid after adsorption to the host cell, at least in part.

Exclusion of superinfecting phage. Wild-type P22 lysogens prevent growth of superinfect-

TABLE 1. *The breakdown of parental phage DNA after infection of starved cells at 41 C*

Phage	Time (min) after infection	Input DNA made acid soluble (%)
c²	0	3.9[a]
	7	30
	32	46
	62	60
	100	60
16⁻ts16.2c2	0	1.4[a]
	8	31
	33	50
	63	64
	101	65
16⁻am-defective particles	0	0.8[a]
	9	34
	34	57
	64	67
	102	70

[a] Phage suspensions were added to medium without cells and treated to precipitate acid-insoluble DNA. The small number of counts found in the supernatant is probably due to the presence of phage which did not precipitate.

ing phage P22 not only by the immunity system but also by an exclusion mechanism (27, 34). Superinfecting P22 does not replicate and neither complements nor recombines with the prophage genome when immunity is released by induction (27). Within a matter of a few minutes after a primary infection with phage P22, superinfecting phage are also excluded from participating in phage production, complementation, and recombination. Exclusion of superinfecting phage P22 by prophage and by a primary infecting phage is due to the same mechanism. Mutants of P22 have been isolated which no longer exclude superinfecting phage; thus, exclusion is under genetic control. These mutants are designated *sie* for super-*i*nfection *e*xclusion (27, 32–34). Since P22 *16⁻ts* cannot carry out an early function on infection, and exclusion occurs early after a primary infection, experiments were undertaken to determine whether P22 *16⁻ts* can establish exclusion at the nonpermissive temperature.

Infections were carried out in *su⁻* strain 18 at 41 C, conditions which are nonpermissive for both mutant phages P22 *16⁻ts* and P22 *12⁻am*. Sequential infections, using one of the mutant phages for the primary infection and normal P22 *c*2 as the secondary infecting phage (15 min after the primary infection), will give infective centers and high yields only if P22 *c*2 can actively participate in the infection. (An infective center is defined as an infected bacterium which gives rise to phage which can form a plaque under specific plating conditions.) However, if the primary infecting phage has established exclusion at the time of superinfection with P22 *c*2, then both infective centers and yields are expected to be low when compared to simultaneous mixed infections. Primary infections carried out with P22 *12⁻amc2* serve as a control. Mutants in gene *12* do not synthesize phage DNA.

A simultaneous mixed infection with P22 *12⁻amc2* and P22 *c*2 gives the expected number of infective centers and a normal phage yield (Table 2, line 1). Sequential infection, with P22 *12⁻amc2* as the primary phage and P22 *c*2 as the secondary phage, gives one-tenth the number of infective centers and one-tenth the number of progeny phage compared to simultaneous infection (Table 3, line 2). That is, by 15 min after infection, P22 *12⁻amc2* has established exclusion and does so in spite of the absence of DNA synthesis. In contrast, P22 *16⁻ts16.1c2* cannot establish superinfection exclusion. There are no differences between the simultaneous and sequential infections (Table 2, lines 3 and 4). In both cases P22 *c*2 can actively participate in the infection.

TABLE 2. *Establishment of superinfection exclusion by a primary infection*[a]

Primary infection	Secondary infection	Infective centers[b]	Phage yield
1. *12⁻amc2sie⁺* × *c2*		2.4×10^8	2.1×10^{10}
2. *12⁻amc2sie⁺*	*c2*	2.8×10^7	2.2×10^9
3. *16⁻ts16.1c2sie⁺* × *c2*	*c2*	2.1×10^8	4.7×10^{10}
4. *16⁻ts16.1c2sie⁺*		2.7×10^8	6.0×10^{10}

[a] Infections were carried out, using multiplicities of 10 for each phage type, in *su⁻* strain 18 at 41 C, the nonpermissive condition for both conditional lethal mutants.

[b] Infective centers were assayed on *su⁻* strain 18 at 41 C.

TABLE 3. *Induction of lysogens by superinfecting phage*[a]

Superinfecting phage	Survivors (%)[b]	Infective centers (%)[c]
1. —	100	1.2
2. *12⁻amc2*	10	37
3. *16⁻am*-defective particles (infection)	112	1.4

[a] 18 (*c⁺sie*) was superinfected with the appropriate phage (MOI = 50). After allowing sufficient time for adsorption and treatment with antiserum, samples were diluted and assayed for survivors and infective centers on *su⁻* indicator bacteria.

[b] Survivors (%) = (number of colonies from sample/number of colonies from uninfected lysogen) × 100.

[c] Infective centers (%) = (number of infective centers from sample/number of colonies from uninfected lysogen) × 100.

Lysogenization of the host cell. Mutations in genes *12* and *18* affect functions necessary for phage DNA synthesis and also decrease the frequency of lysogeny (24). P22 *16⁻ts*, which also does not synthesize phage DNA, was studied to ascertain whether it is capable of lysogenizing the host cell at the restrictive temperature.

Normal frequencies of cell survival are observed after P22 *16⁻ts16.1c⁺* infection at 41 C. However, in contrast to a wild-type infection, these cells do not give rise to lysogenic progeny. That is demonstrated by a study of the segregation pattern of infected cells.

In the control P22 *c⁺* infection at 41 C (Fig. 1A), more than 95% of the cells survive as phage carriers. They begin dividing after a lag of about one generation, and thereafter the total number of cells increases exponentially. The proportion of phage carriers to total cells re-

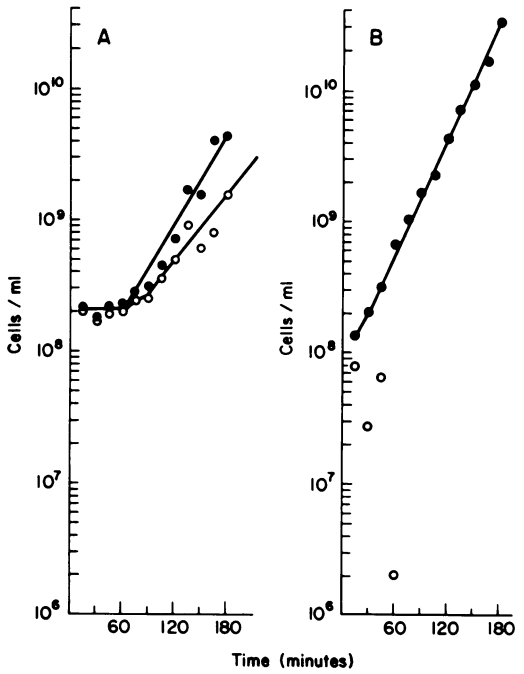

FIG. 1. *Segregation of phage-carrying and sensitive cells at 41 C after infection with either (A) P22 c⁺ or (B) P22 16⁻ts16.1c⁺. Symbols: ●, total number of cells; ○, number of phage-carrying cells.*

mains high for a few divisions, but eventually cells segregate as sensitives and phage carriers. The ratio of phage carriers to total cells remains constant after about four generations. The simplest explanation for these data is that, after an early period of segregation of intracellular phage genomes, prophage integration occurs and thereafter the capacity to produce phage is perpetuated in parallel with growth as expected of stable lysogenic cells (30).

The segregation pattern of cells infected with p22 *16⁻ts16.1c⁺* at 41 C is different from the control infection (Fig. 1B). Although a high proportion of cells survives, only about half are phage carriers. The total number of cells increases logarithmically with no lag after infection, but the absolute number of phage carriers decreases. Thus, P22 *16⁻ts16.1c⁺* does not lysogenize the host.

This result is also different from that described for *int* phage mutants (30) and mutants in genes *12* and *18* (24). Nonpermissive infections with any of these three mutants show a high proportion of the cells surviving as phage carriers, but after some increase in cell titer the absolute number of phage carriers remains constant. The ratio of phage carriers to total cells continues to decrease with each division, indicating the absence of stable lysogeny.

In contrast, during P22 *16⁻ts16.1c⁺* infections, the absolute number of phage carriers decreases. This latter observation is additional evidence for the irreversible inactivation of P*16* after infection with P22 *16⁻ts* at the nonpermissive temperature. In the accompanying paper, evidence is given supporting the contention that P22 *16⁻ts* infections at 41 C are abortive due to irreversible denaturation of P*16* (13).

Mixed infections with P22 *12⁻amc⁺* and P22 *16⁻ts16.1c⁺* in *su⁺* strain 192 at 41 C give some lysogens with *16⁻ts16.1* prophage; therefore, P22 *16⁻ts* can be complemented for lysogeny. *16⁻ts* mutant lysogens, formed either by complementation or by infection with P22 *16⁻ts* at 25 C, are stable at 41 C. It can be concluded that gene *16* product is necessary for the establishment of lysogeny but not for the maintenance of the lysogenic state.

16⁻am-defective particles cannot titrate repressor. Superinfection of a lysogen with sufficiently high numbers of phage overcomes lysogenic immunity. The injected DNA titrates out repressor molecules, repression of phage gene expression is released, and both the prophage and superinfecting genomes are replicated (27, 35). Similar experiments were carried out with *16⁻am*-defective particles (infection) to determine if the infecting phage DNA can titrate repressor. The results should be informative as to the intracellular localization of the released defective particle DNA.

Strain 18 (*c + sie*) was grown at 37 C to a final concentration of 2×10^8 cells/ml and superinfected at multiplicities of 50 with either P22 *12⁻amc2* as a control, or with *16⁻am*-defective particles (infection). Antiserum was added (K = 2), and 5 min later the superinfected lysogens were diluted and assayed for infective centers on *su⁻* indicator bacteria and for surviving bacteria.

Superinfection of a lysogen with P22 *12⁻amc2* induces the majority of the cells, since only 10% of the infected lysogens survive as colony formers (Table 3, line 2). Although 90% of the infected lysogens are killed, not all of these form infective centers with our assay conditions. Only infected cells in which both repressor is titrated out and at least one *am⁺* genome (prophage genotype) is packaged into a mature phage particle will form an infective center on the *su⁻* indicator bacteria. That only 37% of the cells do so is not unreasonable, since each superinfected cell contains 50 *am* genomes for every *am⁺* genome.

16⁻am-defective particles (infection) do not titrate out repressor. After superinfection with *16⁻am*-defective particles, no infective centers above background level appear and no killing is detected (Table 3, lines 1 and 3). The significance of this result with regard to the cellular

localization of the DNA from *16⁻am*-defective particles will be discussed below.

Phage particles either missing P*16* or containing P*16* which is inactivated after infection adsorb to the host cell with normal kinetics and release their DNA from the capsid. However, no other phage functions are detected. There is an early block in the infective process which prevents the expression of any known phage functions after release of phage DNA from the capsid.

Phage with inactive genomes as complementing agent. P*16* is a structural component of the phage head. Complementation for the early function requiring this protein is reported in the accompanying paper (13); thus, P*16* can act in *trans*. A phage particle containing an inactivated genome and functional P*16* may be able to complement for the early P*16* step, even though it is unable to complement for other early functions requiring transcription of the phage genome.

One means of inactivating the virion genome is by UV irradiation. A suspension of P22 *12⁻amc2* was treated with a dose of UV irradiation (15-W G.E. germicidal lamp at a distance of 50 cm for 3 min) resulting in a 3-log reduction in the active phage titer. Even though the plaque-forming ability of these UV-irradiated phage is greatly reduced, it is necessary to show that its DNA cannot be transcribed.

Gene *18* product is necessary for phage DNA synthesis (24). Therefore, if the UV-irradiated phage cannot complement P22 *18⁻* phage, it would most probably be because the UV-inactivated genome cannot be transcribed (in any case, it would not be due to a gene dosage effect). A mixed infection at 41 C with UV-irradiated phage and P22 *18⁻tsc2* does not result in complementation (Table 4, infections 1, 2 and 3), suggesting that gene *18* is not transcribed from the UV-irradiated DNA.

After infection for at least 10 min at 41 C with P22 *16⁻tsl6.2c2*, most of the infected cells cannot form plaques when shifted to 25 C, nor do they lyse (13; Table 4, infection 4). This is due to the absence of functional P*16* early in the infection. However, in the mixed infection with P22 *16⁻tsl6.2c2* and UV-treated phage, all infected cells form plaques when shifted to 25 C and lysis is observed (Table 4, infection 5). This is indicative of complementation by coinfecting UV-irradiated phage for the early step requiring P*16*.

TABLE 4. *Complementation with UV-irradiated phage for the early step requiring P16*[a]

Infection	Infective centers	Progeny active phage/ml
1. UV *12⁻amc2*	9.0×10^5	10^5
2. *18⁻tsc2*	1.3×10^8	7.6×10^7
3. UV *12⁻amc2* × *18⁻tsc2*	1.3×10^8	1.8×10^8
4. *16⁻tsl6.2c2*	1.5×10^7	4.6×10^7
5. UV *12⁻amc2* × *16 tsl6.2c2*	1.2×10^8	2.1×10^8

[a] *su⁺* strain 192 was infected at 41 C with the appropriate phage at a multiplicity of 5 for each genotype. The infected bacteria were plated at 25 C on *su⁺* indicator 10 min after infection.

The active phage titer from the mixed infection is low; the yield of active progeny is only fourfold higher than in a single infection (Table 4, infections 4 and 5). This is expected since newly synthesized wild-type gene *16* protein is necessary for active phage formation, and the UV-irradiated *16⁺ts* phage DNA most likely cannot be transcribed. These data are interpreted to mean that complementation for the late gene *16* step requires a transcribable phage genome, but complementation for the early step does not.

Early step is independent of protein synthesis and immunity control. The following experiments were carried out to determine if the early gene *16* function can take place (i) in the absence of new protein synthesis, and (ii) in a lysogenic host which is immune to superinfection. The design of both experiments is similar. P22 *16⁻tsl6.2c2* infections are initiated at the permissive temperature, but under conditions which prevent phage production. After allowing sufficient time for the early step requiring P*16* to occur (13), the infected cells are shifted to 41 C, the nonpermissive temperature. Simultaneously, the imposed restrictions on phage production are lifted.

P16 action in the presence of chloramphenicol. To inhibit protein synthesis, chloramphenicol (CM), at a final concentration of 100 μg/ml, was added to strain 18 cells growing at 25 C. This concentration of CM prevents phage production when present for the entire incubation period (Table 5, infection 1). However, no amino acid incorporation studies were done to ensure that protein synthesis was completely inhibited.

Brief treatment with CM results in a fourfold drop in the phage yield of a P22 *16⁻ts* permissive infection (Table 5, compare infections 2 and 3). Infections initiated in CM and then shifted to 41 C give yields of active phage and total particles similar to a parallel infection carried out without CM (Table 6, compare infections 4 and 5). Since CM is present during the period the infection is at the permissive temperature, the early gene *16* function must occur under conditions of inhibition of protein synthesis.

TABLE 5. *P16 action in the presence of CM*[a]

	Conditions of infection				Progeny	
	0–20 min		20–180 min			
	Temp (C)	CM	Temp (C)	CM	Active phage/ml	Total particles/ml
1.	25	+	25	+	1.8×10^6	
2.	25	+	25	−	5.4×10^9	
3.	25	−	25	−	1.9×10^{10}	
4.	25	+	41	−	5.9×10^8	8.3×10^9
5.	25	−	41	−	4.2×10^8	9.8×10^9

[a] CM, at a final concentration of 100 μg/ml, was added to strain 18 cells growing at 25 C. Five minutes after the addition of CM, P22 *16⁻ts16.2c2* (MOI = 10) was added to the cell suspension. Ten minutes later free phage were inactivated with antiserum. The infected cells were diluted out of antiserum and into growth medium free of CM at either 25 or 41 C. Parallel infections were carried out in cells not treated with CM. After 180 min, chloroform was added to all infections. The lysates were assayed for active phage, and in some cases also for total particles.

TABLE 6. *Complementation in sequential infections*[a]

Primary infection	Secondary infection	Progeny in yield
1. *12⁻amc2tssie*		4.5×10^7
2. *16⁻ts16.2c2tssie*		3.0×10^7
3. *12⁻amc2tssie* × *16⁻ts16.2c2tssie*		3.5×10^{10}
4. *16⁻ts16.2c2tssie*	*12⁻amc2tssie*	1.3×10^{10}
5. *12⁻amc2tssie*	*16⁻ts16.2ctssie*	1.3×10^{10}

[a] *su⁻* strain 18, at a concentration of 2.5×10^8 cells/ml, was infected at 41 C with the appropriate phage (MOI = 5). Superinfections were carried out 20 min after the primary infection.

P16 action on superinfection of lysogens. The following experiment was done to see whether the P16 step can occur in the presence of immunity.

18 (*12⁻amc2tssie*) was grown to 10^8 cells/ml and then superinfected with P22 *16⁻ts16.2c2* at 25 C. Ten minutes later, the superinfected cells were centrifuged and resuspended in M9CAA medium at 41 C. This temperature thermally induces the lysogen and is nonpermissive for P22 *16⁻ts16.2c2*. In the absence of superinfection, the phage yield is low (9.0×10^5/ml) since strain 18 is nonpermissive for the amber phage. For the superinfected lysogen, total phage production is high (6.0×10^9/ml), with both amber and temperature-sensitive phage produced. An induced prophage cannot complement superinfecting phage for the early step requiring P16 (Hoffman, unpublished results). Therefore, the early P16 function must have taken place at 25 C, the permissive temperature, in the uninduced lysogen. This indicates that the early step is not under immunity control and is independent of most phage transcription and translation.

Complementation for the early P16 step in sequential infections. It is reported in the accompanying paper (13) that a primary P22 *16⁻ts16.2* infection at 41 C can be complemented for the early function by superinfecting phage added at 10 min postinfection. Superinfecting as late as 20 min postinfection still permits rescue of the *16⁻ts* genome (Table 6, compare infections 1–4). The reciprocal sequential infection, P22 *12⁻amc2tssie* as the primary phage and P22 *16⁻ts16.2c2tssie* as the secondary one, was also examined for complementation. In this case, P16 is provided early, and one can ask how long it is available to the superinfecting genome. Complementation for phage production, and therefore for the early P16 function, occurs in this sequential infection. The active phage titer is almost as high as that in a simultaneous mixed infection (Table 6, infections 3 and 5). Similar results were obtained with *16⁻am*-defective particles (infection) as the secondary infecting phage (data not shown).

Complementation for the early gene 16 function can occur in sequential infections in which either the primary or secondary infecting phage is missing functional P16. These findings permit the conclusions that no irreversible damage occurs to the genome of the P16⁻ phage during the first 20 min of a nonpermissive infection and that the *trans* effect of P16 persists for at least 20 min.

P16 dosage experiments. Phage missing functional P16 can be complemented for the early step requiring this protein. In all cases of complementation so far examined, the multiplicity of the P16⁺ phage was greater than or equal to the multiplicity of the P16⁻ phage. Mixed infections were carried out to determine whether complementation is affected by the relative amounts of P16 to the number of phage genomes in the infected cell. Since the *16⁻am*-defective particles (infection or induction) contain no P16, the amount of this protein relative to the number of phage genomes can be manipulated by varying the MOI of both P16⁺ phage and *16⁻am*-defective particles.

Criteria for determining complementation. Mixed infection with *16⁻am*-defective particles (infection) and P22 *18⁻tsc2* were initiated in *su⁺* strain 192 at 25 C, conditions which are permissive for both temperature-sensitive and amber mutant phage. *16⁻am*-defective particles, however, are not productive in any host at

any temperature unless P16 function is provided by another phage. Therefore, in these infections, ts^+ phage will be produced and infective centers will appear on su^+ indicator bacteria at 41 C only if complementation for the early P16 function occurs. Two measurements of complementation were used. One measurement is the fraction of mixedly infected cells which form infective centers on su^+ indicator bacteria at 41 C. This fraction, the number of infective centers at 41 C on su^+ indicator bacteria divided by the number of mixedly infected cells, is referred to as P_c. P_c values near 1 indicate that *16⁻am*-defective particles are complemented for the early P16 reaction in most mixedly infected cells. P_c values significantly less than 1 are indicative of the failure of P22 *18⁻tsc2* to complement the *16⁻am*-defective particles. Secondly, the percentage of ts^+ phage in the total yield from mixedly infected cells reflects the marker contribution of the genomes carried in the *16⁻am*-defective particles and thus is another measure of complementation for the P16 function.

Complementation is sensitive to the input ratio of defective particles and phage. As demonstrated (13) in the infective center assay for defective particles, mixed infection of a su^+ host at 25 C with high multiplicities of P22 *18⁻tsc2* and low multiplicities of *16⁻am*-defective particles results in an infective center titer equal to the defective particle titer. By definition, P_c for these infections is equal to 1 since the number of mixedly infected cells, the denominator, is determined by the number of infective centers, the numerator (Table 7, infections 1 and 2). Infections with low multiplicities of both P22 *18⁻tsc2* and *16⁻am*-defective particles, such that mixedly infected cells contain only one copy of each, give P_C values close to 1 and high percentages of ts^+ progeny (Table 7, infections 3 and 4). Thus, one phage can complement one defective particle for the early step.

Cells infected with one copy of P22 *18⁻tsc2* and increasing multiplicities of *16⁻am*-defective particles give decreasing P_c values (Table 7, infections 5, 6, and 7), and decreasing percentages of ts^+ phage in the yield. In other words, the efficiency of complementation for the early P16 step in cells infected with one P22 *18⁻tsc2* phage and more than one *16⁻am*-defective particle decreases as the number of *16⁻am*-defective particles increases.

The idea that the amount of P16 relative to the total number of phage affects the degree of complementation is illustrated, in addition, by the fact that for high multiplicities of *16⁻am*-defective particles, raising the multiplicity of P22 *18⁻tsc2* increases the level of complementation, as measured by both P_c and the percentage of ts^+ progeny (Table 7, infections 8–15).

Figure 2 is a plot of the multiplicity ratio, P22 *16⁻am*-defective particles/P22 *18⁻tsc2*, versus P_c. For a multiplicity ratio less than one, fewer defective particles than phage, P_c is approximately 1. P_c drops precipitously when the multiplicity ratio exceeds one. The presence of active phage in the *16⁻am*-defective particle (infection) suspension accounts for the observation that P_c never falls below 0.04.

TABLE 7. *Effect of multiplicity on complementation for the early step*

	MOI		P_c[a]	ts^+ phage in yield (%)[b]
	P22 *18⁻tsc2*	*16⁻am*-defective particles		
1.	5	0.1	1[c]	20
2.	5	0.5	1[c]	22
3.	0.5	0.1	1.3	42
4.	0.5	0.5	0.7	27
5.	0.5	3	0.1	5
6.	0.5	7	0.06	3
7.	0.5	13	0.05	2
8.	0.5	5	0.04	4
9.	5	5	0.5	23
10.	10	5	0.7	32
11.	0.5	10	0.05	2
12.	5	10	0.5	10
13.	10	10	0.6	22
14.	5	20	0.2	5
15.	10	20	0.5	10

[a] P_c = number of infective centers at 41 C on su^+ indicator/number of mixedly infected cells.

[b] The number of plaques on su^+ indicator bacteria at 41 C, expressed as a percentage of the total yield from mixedly infected cells.

TABLE 8. *Frequency of transduction of bacterial markers*[a]

Phage	Method of preparation	Transductants per 10^8 particles[b]	
		hisG201	*proC90*
1. *c1*	Infection	0.25	0.12
2. *16⁻amc1*-defective particles	Infection	$<10^{-3}$	$<2 \times 10^{-4}$
3. *c2ts*	Induction	1.3	7.0
4. *16⁻ts16.2c2ts*-defective particles	Induction	0.08	0.4

[a] CsCl-purified phage suspensions, prepared by infection or induction, were assayed for transducing particles using auxotrophic recipients lysogenic for P22 *sie1*. Only complete transductants were scored.

[b] Absolute phage particle concentrations were determined by optical density at 260 nm.

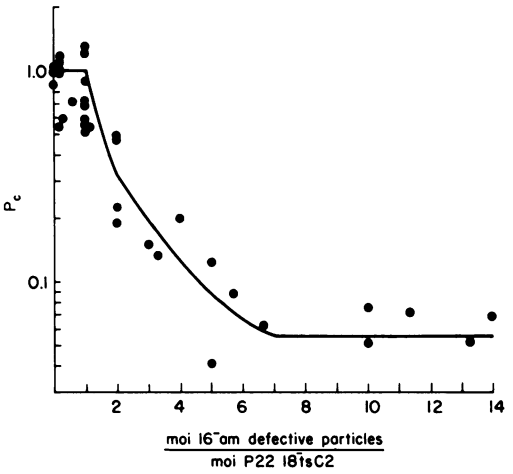

FIG. 2. *The effect of the multiplicity ratio (16⁻am-defective particles/P22 18⁻tsc2) on complementation for the early 16 function. These multiplicities are for infected cells. For example, if a multiplicity of 0.1 is used, then the multiplicity for the infected cell is one. P_c = The number of infective centers at 41 C on su⁺ indicator/the number of mixedly infected cells.*

In all these mixed infections, P22 *18⁻tsc2* progeny production is normal. This is shown by the fact that (i) the number of infective centers at 25 C, plated on either *su⁺* or *su⁻* indicator bacteria, is always equal to the number of P22 *18⁻tsc2*-infected cells, and (ii) the number of temperature-sensitive progeny in the yield is always high (data not shown).

Complementation for the early step requiring P*16* depends on the ratio of defective particles to P*16⁺* phage. One phage complements one defective particle efficiently, but complements two or more defective particles poorly. Thus, the presence of two defective particles hinders complementation for either one by a single P*16⁺* phage. In general, if there are more defective particles than phage, complementation is inhibited. As the number of *16⁻am*-defective particles relative to phage increases, the extent to which P*16⁺* phage can complement the defective particles drops. In all instances, the replication of P22 *18⁻tsc2* is not inhibited by any number of coinfecting *16⁻am*-defective particles.

Specificity of the early P16 function. In the absence of P*16* activity, infecting phage do not appear to initiate any synthetic processes leading to phage production. P22 generalized transducing particles contain primarily bacterial DNA and little or no phage DNA (11). The mechanisms of injection of this DNA into the bacterial cell is presumed to be the same as that for phage DNA. It can be asked whether P*16* activity is needed for successful transduction. Another question is whether phage L, a related but heteroimmune phage (4, 5, 7), can complement P22 *16⁻am*-defective particles. Phage L and P22 are closely related such that recombination occurs between the two.

Transduction requires functional P16. A comparison of the transducing activity of P*16⁺* and P*16⁻* phages for bacterial *proC* and *hisG* markers was carried out. No transductants were detected for either the *proC* or *hisG* markers with *16⁻am*-defective particles (infection) as donor, although normal frequencies were obtained for P22 *c*1 (Table 8, lines 1 and 2). In phage suspensions from thermal induction of 18(*16⁻ts16.2c2ts*), only 1 out of 30 particles has P*16* activity. The frequencies of transduction for both the *proC* and *hisG* markers are about 15-fold lower using this suspension as donor than the one from thermal induction of 18(*c2ts*), in which P*16* activity is present in all particles (Table 8, lines 3 and 4). It can be concluded that particles which are missing P*16* do not transduce the host cell.

The question can be asked if the failure of P*16⁻* particles to transduce is a direct result of the absence of P*16*, or due to the absence of bacterial DNA in these defective particles. To distinguish between these two possibilities, the ability of P22 grown on *hisG* auxotrophs to complement *16⁻am*-defective particles (infection) for transduction of the *hisG* marker was examined. The frequency of transductants is 0.4 per 10^6 particles for donor P22 *c*1 grown on a *hisG* prototrophic host. However, as expected, no transductants were detected if the donor was obtained from a *hisG* auxotrophic host (<0.001 per 10^6 particles). No transductants were observed after infection with *16⁻am*-defective particles (infection) produced in the wild-type host (<0.005 per 10^6 particles). However, a mixed infection using P22 *c*1 from a *hisG* auxotrophic

TABLE 9. *Complementation between phage L and 16⁻am-defective particles (infection)*[a]

Phage	MOI	Infective centers
1. *16⁻am*-defective particles	0.3	1.0×10^4
2. L	10	$<10^5$
3. *18⁻tsc2*	10	$<10^5$
4. *16⁻am*-defective particles × L	0.3:10	$<10^5$
5. *16⁻am*-defective particles × *18⁻tsc2*	0.3:10	2.2×10^7

[a] *su⁺* strain 192 was infected at 25 C. Infective centers were assayed on 192(L) at 41 C. Neither P22 *18⁻tsc2* nor phage L form plaques under these plating conditions.

host and *16⁻am*-defective particles (infection) resulted in normal frequencies of transductants (0.4 per 10^6 particles). This clearly demonstrates that the *hisG*$^+$ marker is present in the *16⁻am*-defective particle (infection) DNA. Thus, failure of *16⁻am*-defective particles (infection) to transduce the host cell is due to the absence of P*16* activity. Thus, the early P*16* requirement is not unique to infecting phage DNA, but is also essential for infecting bacterial DNA.

Complementation for the early step with phage L. Mixed infections with phage L (MOI = 10) and *16⁻am*-defective particles (infection, MOI = 0.3) fail to show any infective centers at 41 C on 192 (L) indicator bacteria (Table 9, infections 1, 2, and 4). P22 amber mutants form plaques on 192(L) indicator, but phage L does not. In contrast, a control infection with P22 *18⁻tsc2* (MOI = 10) and *16⁻am*-defective particles (infection, MOI = 0.3) gives more than a 3-log increase in infective centers (Table 9, infections 1, 3, and 5).

The failure of phage L to complement for the early P*16* function can be explained by any of the following possibilities. (i) Phage L has no protein analogous to P*16*. (ii) The action of P*16* is phage specific. The phage L protein analogous to P*16* cannot substitute for P*16*. (iii) The P*16* analog in phage L cannot act in *trans*. At the moment, no distinction can be made between these possibilities. However, analysis of the protein components of the L virion by high resolution sodium dodecyl sulfate-acrylamide gel electrophoresis reveals a banding pattern similar to the one from P22 virions (data not shown). L virions have a minor protein component with a molecular weight close to that of P*16*, suggesting that there is a protein analogous to P*16*.

DISCUSSION

P*16* is a head protein of phage P22 which has no essential role in phage assembly, but which is required in the following cycle of infection (9, 13, 16). P22 *16⁻ts* phage contain thermolabile P*16* which is inactivated at high temperature on infection. Temperature shift experiments with such phage show that the virion-carried P*16* must act within the first 10 min after infection at 25 C. P*16* is also required late in infection in order that the progeny particles be infectious. Defective particles missing P*16* are produced (i) during nonpermissive P22 *16⁻am* infection, (ii) when a P22 *16⁻ts* infection initiated at 25 C is shifted to 41 C, and (iii) on induction of a gene *16* mutant lysogen under nonpermissive conditions (13). Thus, no functional P*16* is available on infection with P22 *16⁻ts* at high temperature or with defective particles under any conditions.

Infections in the absence of functional P16. The following features of infection in the absence of functional P*16* are well established: (i) adsorption proceeds with normal kinetics and (ii) phage DNA is at least partially released from the capsid; (iii) the host cells are not detectably affected; they continue to grow and divide with the same kinetics as uninfected cells (6; Hoffman, Ph.D. thesis); (iv) no change in the rate of DNA synthesis in the infected culture is observed, suggesting that no phage DNA is synthesized (6; Hoffman, Ph.D. thesis); (v) the parental phage DNA does not associate with the replication complex (23); (vi) these infections do not result in the transcription of any phage-specific RNA, as determined by RNA-DNA hybridization (E. N. Jackson, personal communication); (vii) superinfection exclusion is not induced; (viii) no phage protein synthesis is detected (15); (ix) two alterations in enzymatic activity are detected early in P22 infections—a loss of host DNase activity specific for denatured DNA (15) and an increase in phage-specified DNase activity specific for native DNA (36); infections in the absence of P*16* activity fail to show either of these effects; and (x) the host cannot be lysogenized. Thus, in the absence of functional P*16*, there is a block at an early step in the infective process which prevents the expression of any phage functions following at least partial release of phage DNA from the capsid.

The nature of the early gene 16 function. This section will summarize all of the data about the early gene *16* function; that is, the activity carried out by the virion-carried P*16*.

The activity of P*16* is required only within the first 10 min after infection at 25 C for the infection to be viable. This argues against the P*16* function being similar to the N function of bacteriophage λ.

The early P*16* reaction can take place in the presence of chloramphenicol and, therefore, probably does not require protein synthesis for its expression.

The early P*16* reaction occurs in lysogenic cells. The reaction is not under immunity control.

P*16*$^-$ particles can be complemented for the early P*16* activity. Phage inactivated by UV irradiation as well as viable phage can be the complementing agent. Thus, P*16* can act in *trans* and gene expression by the complementing phage is not required.

Complementation for the early P*16* reaction occurs when the primary infecting phage is P*16*$^+$ and the secondary infecting phage is P*16*$^-$.

That is, P*16* from the primary infection, or the consequence of its action, is still available to superinfecting P*16*$^-$ phage very late into the latent period.

P*16*$^-$ primary infections can be complemented, and the P*16*$^-$ genome can be rescued, by superinfecting P*16*$^+$. Thus, in the absence of P*16*, the infection is blocked at some point in the normal sequence of events, and the phage genome is not irreversibly damaged. In the absence of P*16*, the released DNA is not susceptible to host-induced damage in normally metabolizing cells. This finding eliminates a protecting role for P*16*.

Efficient complementation results only when the number of infecting P*16*$^+$ phage is equal to or greater than P*16*$^-$ phage. In all instances, the production of P*16*$^+$ phage is not affected by the coinfecting P*16*$^-$ particles. P*16*$^+$ phage production is normal whether or not complementation for the early gene *16* function has taken place.

These data suggest that P*16* acts initially in *cis*. Yet the capacity of a P*16*$^+$ virion to complement a P*16*$^-$ particle shows that the protein can also act in *trans*. If the action of P*16* is to cause a generalized change in the host cell, such as an alteration in large areas of the host membrane or inactivation (or modification) of host cell enzymes, then a single P*16*$^+$ particle should initiate these changes. Any number of other particles, whether P*16*$^+$ or P*16*$^-$, should then be able to effectively infect the cell. This is clearly not the case. Thus, all models of P*16* action which suppose a generalized change in the infected cell are unlikely. P*16* action must be localized, either affecting only a small region of the cell or acting directly on a phage or phage genome. If the latter is the case, it could be further argued that P*16* must be released from the capsid to accomplish complementation. One explanation for the finding that an excess of defective particles prevents complementation for any of them is that there is dilution of P*16* among the many DNA molecules or phage particles.

Generalized transduction requires functional P*16*. This shows that the early gene *16* step in the infective process is not specific for phage DNA. Ebel-Tsipis et al. (12) have shown that transducing DNA is integrated into recipient bacterial DNA in fully conserved, double-stranded form. Therefore, the initial events after transduction do not require replication of the transducing DNA. The fact that transduction requires P*16*, but not replication of the transducing DNA, may be taken as evidence that the early P*16* function is not directly involved with replication of P22 DNA.

Superinfecting DNA from P*16*$^-$ phage does not titrate repressor. This may be because the released phage DNA either is not available to the repressor molecules, or is in a conformation which prevents repressor binding. Thus, the P*16* function must occur before phage DNA can bind repressor.

Induction of gene *16* mutant lysogens under nonpermissive conditions proceeds with the expression of early and late genes, resulting in defective particle production and cell lysis. Thus, the early P*16* requirement is specific for infection. That the induced prophage genome bypasses the requirement for P*16* demonstrates that P*16* is not an integral part of the phage replication machinery and cannot have an early regulatory role or an essential enzymatic activity for phage production.

Finding that parental DNA does not appear in intermediate I in the absence of functional P*16*, Levine et al. (23) suggested that P*16* may be active in binding parental DNA to the phage replication machinery (8). This now appears most unlikely for the following reasons: P*16* is not necessary for phage DNA synthesis on induction; the early P*16* activity and the binding of parental DNA to intermediate I are unique and separable events in infection, since the former is not under immunity control and the latter is (23); and since transducing DNA is integrated into the bacterial chromosome without being first replicated (12), it is not likely to be associated with the replication machinery. Yet P*16* function is required for transduction.

Thus, the following types of functions for P*16* have been ruled out: (i) protection of viral DNA from host-induced damage; (ii) generalized alteration in the cell such as a change in the cell membrane or inactivation (or modification) of host cell enzymes; (iii) an early regulatory function; (iv) an enzymatic activity required for phage production; (v) P*16* being an integral part of the phage replication machinery; (vi) and an involvement of P*16* in binding parental phage DNA to the replication complex.

Possible functions for P16. Little is known about the early events in a P22 infection, particularly about the steps between adsorption of the virion to the host cell and association of the parental phage DNA with intermediate I. The P*16* function, which is required only on infection, occurs after at least partial release of the DNA from the capsid and prior to association with the replication machinery. The role of P*16* may be one of the following: (i) completing release of DNA from the capsid; (ii) bringing the released DNA to the appropriate compartment in the cell; (iii) removal of virion proteins which are bound to the injected DNA allowing transcription and replication; and (iv) conver-

sion of injected DNA to a conformation which renders it active. Each of these possibilities will be discussed.

(i) Phage DNA is susceptible to host degradative enzymes in P*16*$^-$ infections of starved cells. Thus, in the absence of P*16*, the DNA is at least partially released from the capsid into the cell. There is no direct evidence that the DNA is completely released and disengaged from the capsid. This final process may require P*16*. The *trans* activity of P*16* and the dependence of successful complementation on the input multiplicities imply that this protein is released from the capsid.

There are precedents for two-step transfer of phage DNA into the host cell. Release of the phage T5 DNA molecule from the infecting particle into an *Escherichia coli* cell is divided into two stages: transfer of an initial protein (the first-step-transfer or FST section), amounting to about 8% of the whole molecule, followed by transfer of the remainder (17, 20, 25). The second transfer is under control of FST-DNA genes (18, 19). This is a case in which the release of most of the phage DNA from the particle requires a phage-specified function dependent on newly synthesized proteins in the host.

(ii) P*16* may be involved in bringing the released DNA to the appropriate compartment within the cell. The DNA must either attach to or pass through the host membrane to reach the proper cellular site necessary for subsequent activity.

There is no direct evidence that the released phage DNA is transported beyond the cytoplasmic membrane in the absence of P*16*. The DNA may be in the periplasmic space, between the cell wall and membrane, and the action of P*16* is required to get it beyond this barrier.

(iii) There may be virion proteins bound to the infecting DNA. Unless they are removed, transcription and replication are prevented. P*16* may act to clear the DNA of these proteins, rendering it infectious. The bound proteins may be residual capsid proteins which serve no specific function or, alternatively, these proteins may have a specific role to play after DNA release.

(iv) P*16* may produce an essential structural alteration in the released phage DNA. The structural change may be in the primary structure, such as nicking a strand, which might make sites available to particular enzymes. Another possibility is that the secondary or tertiary structure of the P22 DNA is changed by the action of P*16*. The T4 gene-*32* protein (2, 3) is required in stoichiometric amounts for replication of T4 DNA in vivo (28). Huberman and Kornberg (14) suggest that *32*-protein stimulates T4 DNA polymerase by removing inhibitory secondary structures from the template DNA strand.

At the moment, we can offer no basis on which to distinguish these alternatives.

P*20* is another P22 head protein which plays no direct role in particle assembly, but which is necessary for infectivity. P*20*$^-$ defective particles are produced in P22 *20*$^-$*am* nonpermissive infection (9). Whether P*16* and P*20* act in concert or independently in infection is not now known.

There appears to be a situation for the coliphage T7 analogous to that which we have described for P22. Nonpermissive infections with conditional mutants in either gene *7* or gene *13* of T7 result in the formation of DNA-containing particles which lack either protein specified by these loci (31). They appear normal in electron micrographs, but are not infectious and cannot be activated in vitro. Complementation of these noninfectious particles in mixed infections has not yet been tried (Studier, personal communication.)

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-15419-08 from the National Institute of General Medical Sciences. One of us (Barbara Hoffman) was a National Institutes of Health predoctoral fellow supported by Public Health Service training grant GM-00071 from the National Institute of General Medical Sciences.

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