

# Bacteriophage P22 Virion Protein Which Performs an Essential Early Function

## I. Analysis of $16^{-ts}$ Mutants

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The product of gene *16* of phage P22, *P16*, is a head protein. *P16* does not play an essential role in phage assembly since particles formed without this protein appear normal by electron microscopy examination (Botstein et al., 1973). *P16* is essential when the particle infects a cell in the following cycle of infection (Botstein et al., 1973; King et al., 1973). We have characterized a mutant of P22 carrying a temperature-sensitive allele of gene *16*. This mutant has previously been referred to as P22 $25^{-ts}$  (Levine et al., 1970, 1972) and P22 $X^{-ts}$  (Bezdek and Soska, 1970, 1973). P22  $16^{-ts}$  behaves as an early mutant at the nonpermissive temperature. Temperature shift experiments show that *P16* of the infecting virion acts within the first 10 min at 25 C and that gene *16* product is required late in the latent period for incorporation into infectious phage. Induction does not require *P16* for the production of particles. Particles produced either in a P22  $16^{-ts}$  thermal shift-up infection or after induction of  $16^{-ts}$  lysogens at 41 C are missing *P16* and are, therefore, defective. *P16* in P22  $16^{-ts}$  virions formed at the permissive temperature appears to be heat labile; it is inactivated after infection at 41 C. A simple assay for defective particles based on a complementation test is described.

A morphogenetic pathway for head assembly and encapsulation of DNA has been described for the temperate phage P22 (6, 13). Using amber mutants in genes *16* and *20*, two proteins were detected which are normal constituents of the phage head. These proteins, *P16* and *P20*, are found in the prohead structure, an early intermediate in the formation of P22 heads. Normal appearing phage particles are produced during nonpermissive  $16^{-am}$  and  $20^{-am}$  infections. These particles are missing either *P16* or *P20* and are noninfectious. Botstein et al. (6, 13) concluded that these two virion proteins are dispensable for assembly of the P22 virion but are required for a critical function in the following cycle of infection.

It would be advantageous to have temperature-sensitive (*ts*) mutants to understand the functions carried out by *P16* and *P20* that are essential to successful infections. Temperature shift experiments carried out with these mutants could yield information on the time of action of the proteins and the conditions required for their activity. It should be possible to separate the early activities required for viable phage production from those that occur late in

infection (assembly). Temperature-sensitive mutants for gene *16* have been found and are described in this paper.

P22  $16^{-ts}$  phages conform to the expected phenotype for mutant phage containing thermo-labile *P16* in the virion. These phages are not infectious at the nonpermissive temperature. No phage functions are induced, phage DNA synthesis is not initiated, and no progeny particles are produced. Since *P16* is part of the virion, the early requirement for this protein should be specific for infection, but it should not be needed for particle production on induction of a lysogen. That this is the case is demonstrated by the finding that phage DNA synthesis does occur, and progeny particles are produced after induction of a  $16^{-ts}$  lysogen at high temperature. However, like the nonpermissively produced  $16^{-am}$  particles, these particles are not able to initiate a subsequent infection. Infections with P22  $16^{-ts}$ , initiated at permissive temperature and shifted after a few minutes to nonpermissive temperature, give results similar to those found after induction. DNA synthesis and progeny particle production take place, but the particles are again noninfectious at any temperature. All these noninfectious particles are missing *P16*. Evidence is presented suggesting that P22  $16^{-ts}$  virions, formed during per-

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missive infection, contain thermolabile P16.

In the accompanying paper, experiments are presented and discussed dealing with the early viral function of P16.

#### MATERIALS AND METHODS

**Bacteriophage strains.** Wild-type phage P22 and the clear mutants P22 *c1-7* and P22 *c2-5* have been previously described (14, 17). P22 *c1* and *c2* mutants cannot lysogenize the host in single infections; all infected cells lyse. The mutant P22 *c2-ts30* was obtained from M. Gough. P22 *c2-ts* infections at 41 C are like P22 *c2* infections, but this mutant phage can stably lysogenize the bacterium at 25 C. These lysogens induce at high temperature.

Phage with conditional lethal mutations in either gene 12 or gene 18 fail to synthesize phage DNA at nonpermissive conditions. If these mutants are also either *c1* or *c2*, they turn off host DNA synthesis and kill the host (18). Gene 5 mutant infections result in no head production, but normal quantities of free tail parts are produced (5). The gene 13 mutant delays lysis and allows for continued phage production (4). Morphologically normal but noninfectious particles are produced during a gene 20 or gene 16 mutant nonpermissive infection (6, 13).

The temperature-sensitive conditional lethal mutant P22 18<sup>-ts18.1</sup> and the amber conditional lethal mutants P22 5<sup>-amN114</sup> and P22 16<sup>-amN121</sup> were isolated in this laboratory from wild-type phage P22 treated with 1-methyl-3-nitro 1-nitrosoguanidine. P22 13<sup>-amH101</sup> and P22 20<sup>-amN20</sup> were obtained from D. Botstein. P22 16<sup>-amH60</sup> and P22 12<sup>-amH80</sup> were given to us by Kolstad and Prell. P22 16<sup>-ts16.2</sup> was obtained from Bezdek, who refers to it as P22 *tsX* (1, 2). In previous publications (4, 15, 16) this mutant was called 25<sup>-ts25.2</sup>. Evidence is presented below that mutants previously designated as in gene 16 and gene 25 are noncomplementing and form one locus. Gene 16 has been retained as the designation.

With the exception of amber mutations in gene 16, only one mutant allele for each gene was used in this report. Phage carrying these mutations thus are referred to by gene number and the type of conditional lethal, *ts* or *am*. When both 16<sup>am</sup> mutants were used, as in the complementation tests, the particular alleles are given. However, for all other experiments, only P22 16<sup>-amN121</sup> was utilized and it is referred to as P22 16<sup>-am</sup>.

**Bacterial strains.** A derivative of *Salmonella typhimurium* LT2 cured of the PB<sub>1</sub> phage (22) was used as the sensitive wild-type strain and is designated strain 18. Strain 192 is an LT2 amber suppressor strain (*su*<sup>+</sup>) obtained from D. Berkowitz. Strain 18 is *su*<sup>-</sup>. Lysogens 18 (*c2-ts*) and 18 (*c2-ts 16 ts*) were isolated after infection at 25 C.

**Media.** M9CAA medium (21), tryptone agar (7), soft agar for top layers (14), and buffered saline (14) have been previously described.

**Purification of phage stocks in cesium chloride.** Phage were purified according to the procedure given by Botstein (3).

**Tail production and purification.** Strain 18, grown to a concentration of 2 × 10<sup>8</sup> cells/ml, was

infected at a multiplicity of infection (MOI) of 5 with P22 5<sup>-am 13 amcl</sup>. No head-like structures are seen in gene 5 mutant lysates; however, tail production is normal (5, 6, 13). The gene 13 mutation delays lysis but allows for continued synthesis of phage components and phage production. The double mutant permits increased accumulation of tail parts. Three hours after infection, the cells were concentrated by low-speed centrifugation and suspended in buffered saline. The cells were ruptured by sonic treatment and the addition of chloroform, and cell debris was removed by low-speed centrifugation. Contaminating phage were removed by centrifugation at 17,000 rpm for 100 min. Ammonium sulfate to 70% saturation was added to the supernatant at 0 C with continuous stirring. The mixture was allowed to stir for an additional 20 min. The precipitate, which contained tails, was collected by centrifugation, resuspended in a few milliliters of buffered saline, and dialyzed overnight at 4 C against 0.03 M acetate buffer (pH 5.0). The newly formed precipitate was removed by centrifugation and the supernatant was kept at 79 C for 15 min to inactivate contaminating phage. The precipitate which formed was removed by centrifugation. A high concentration of tails was found in the supernatant (V. Israel, personal communication; 6).

**Tail assay.** Tail titers were determined according to the methods described by Israel et al. (11).

**Thermal induction of lysogens.** Lysogens carrying *c2-ts* mutant prophage are stable when grown at 25 C. When these lysogens reached a concentration of 2 × 10<sup>8</sup> cells/ml, the cells were pelleted by low-speed centrifugation and suspended in supplemented M9 medium prewarmed to 41 C to initiate thermal induction.

**Addition of tails.** Since phage particles arising from induction of lysogens are always deficient in tails (10), tails were routinely added to the lysates in an amount sufficient to activate all tailless phage. Also, tail production is temperature sensitive, even in wild-type infections (10). Therefore, tails were routinely added to all lysates from infections carried out at 41 C. Control reactions with known concentrations of phage heads were always run simultaneously.

**Measurement of the rate of DNA synthesis.** The rate of DNA synthesis was estimated by the incorporation of [<sup>3</sup>H]thymidine into acid-insoluble material during a 1-min pulse (21).

**Discontinuous SDS-polyacrylamide gel electrophoresis of phage particle proteins.** Phage particles were purified in cesium chloride and dialyzed against 1,000 volumes of buffered saline. Two volumes of this phage suspension were added to one volume of a mixture of sodium dodecyl sulfate (SDS; 6%), glycerol (30%), 2-mercaptoethanol (15%), bromophenol blue (0.006%), and trisphosphate buffer (pH 7.1, 0.03 M). The mixture was boiled for 5 min to dissociate the virion into its component proteins. Cylindrical gels were prepared and run using the SDS-discontinuous buffer system for acrylamide gel electrophoresis as described by Maizel (19). The gels consisted of a 10-cm resolving gel (7.5% acrylamide) and a 2.5-cm spacer gel (3% acrylamide). Samples containing 10<sup>11</sup> particles in a volume no larger than

0.1 ml were carefully layered onto the top of the gels. Samples were run using fixed voltage (100 V) until the dye reached the bottom of the tube. The gels were fixed with 20% sulfosalicylic acid for 16 to 24 h, stained with Coomassie blue for 3 h, and destained by repeated washings with 7% glacial acetic acid.

## RESULTS

**Genes 16 and 25 form one cistron.** P22 particles missing P16 are obtained after infection of *su*<sup>-</sup> host cells with P22 16<sup>-</sup>*am*. Botstein et al. (6) and King et al. (13) report that these particles are not viable and conclude that P16 is necessary to carry out a function early in the infective process. If this is so, infections at high temperature with virions containing thermolabile P16 should also be blocked early in the infective process. In an attempt to find such phage, we carried out complementation tests with various phage mutants known to have an early defect, failure to synthesize phage DNA.

Mixed infections with P22 25<sup>-</sup>*ts* (see above) and the two different P22 16<sup>-</sup>*am* mutants clearly show that these mutants do not complement for phage production. The yields from mixedly infected cells at 41 C in a *su*<sup>-</sup> host are not significantly different than those from single infections (Table 1, compare infections 1, 2, 6; 1, 3, 7). In contrast, P22 18<sup>-</sup>*tsc2*, an early temperature-sensitive mutant which complements P22 25<sup>-</sup>*tsc2*, also complements P22 16<sup>-</sup>*amN121* (Table 1, compare infections 3, 4, 8). In addition, P22 20<sup>-</sup>*am*, a late amber mutant, also complements P22 25<sup>-</sup>*tsc2* (Table 1, compare infections 1, 5, 9). According to the complementation tests, mutants assigned to genes 16 and 25 appear to be in the same complementation group. Gene 25 mutant phage have been described in a number of publications (1, 2, 4, 15, 16). These mutant phage will now be referred to as gene 16 mutants.

Data will be given in the remainder of this

TABLE 1. Complementation between P22 25<sup>-</sup>*tsc2* and amber mutants<sup>a</sup>

Infections	Active phage in lysate
1. 25 <sup>-</sup> <i>tsc2</i> .....	2.1 × 10 <sup>7</sup>
2. 16 <sup>-</sup> <i>amH60c1</i> .....	1.4 × 10 <sup>6</sup>
3. 16 <sup>-</sup> <i>amN121c1</i> .....	8.7 × 10 <sup>6</sup>
4. 18 <sup>-</sup> <i>tsc2</i> .....	6.2 × 10 <sup>7</sup>
5. 20 <sup>-</sup> <i>am20c2</i> .....	3.0 × 10 <sup>6</sup>
6. 25 <sup>-</sup> <i>tsc2</i> × 16 <sup>-</sup> <i>amH60c1</i> .....	7.2 × 10 <sup>6</sup>
7. 25 <sup>-</sup> <i>tsc2</i> × 16 <sup>-</sup> <i>amN121c1</i> .....	5.4 × 10 <sup>7</sup>
8. 18 <sup>-</sup> <i>tsc2</i> × 16 <sup>-</sup> <i>amN121c1</i> .....	2.3 × 10 <sup>10</sup>
9. 25 <sup>-</sup> <i>tsc2</i> × 20 <sup>-</sup> <i>am20c2</i> .....	9.6 × 10 <sup>9</sup>

<sup>a</sup> *Su*<sup>-</sup> strain 18, at a concentration of 2 × 10<sup>9</sup> cells/ml, was infected at 41 C with phage at a multiplicity of 5 for each genotype.

paper which show that mutant phage P22 16<sup>-</sup>*ts* conforms to the expected phenotype for phage containing thermolabile P16.

**Thermal induction of 16<sup>-</sup>*ts* mutant lysogens.** It is not expected that virion proteins would be involved in the early events after induction of lysogens. Induction of 16<sup>-</sup>*ts* lysogens at the nonpermissive temperature was carried out to determine if there is an early requirement for the product of gene 16.

Lysogens 18 (16<sup>-</sup>*ts c2-ts*) and 18 (*c2-ts*) were grown to 10<sup>8</sup> cells/ml at 25 C, concentrated by centrifugation, and suspended in prewarmed (41 C) M9CAA medium. This treatment results in induction of both lysogens, as measured by cell lysis. The lysis curve of the heat-treated 18 (16<sup>-</sup>*ts c2-ts*) closely parallels that of the control (Fig. 1A). Both curves show a rise in absorbance followed by a drop, indicating cell lysis.

The rates of DNA synthesis for the thermally induced lysogens were determined (Fig. 1B). For both 16<sup>-</sup>*ts* and 16<sup>+</sup> lysogens, there is a depression in the rate between 10 and 20 min followed by a rise, which peaks after 40 min, followed by a rapid fall. Thus, the pattern of DNA synthesis after thermal induction of 16<sup>-</sup>*ts* lysogens is similar to that of 16<sup>+</sup> lysogens.

The yield of active phage after thermal induction of 16<sup>-</sup>*ts* lysogens is low, approximately 5%, compared to that of 16<sup>+</sup> lysogens (Table 2). Two possible reasons for this can be put forward: (i) induction occurs, but particle production is

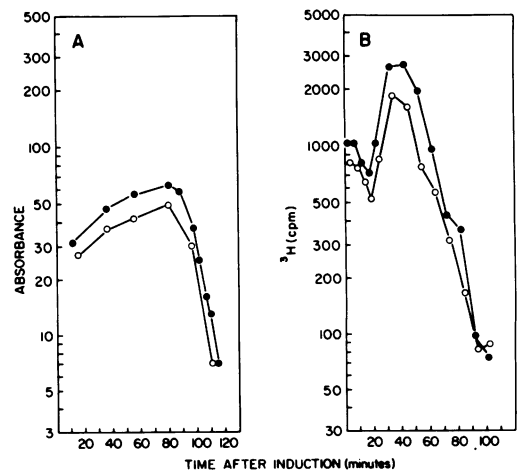


FIG. 1. Thermal induction of 18 (*c2ts*) and 18 (16<sup>-</sup>*ts c2ts*) at 41 C as followed by absorbance (A) and rate of incorporation of [<sup>3</sup>H]thymidine into acid-precipitable DNA (B). One-minute pulses of [<sup>3</sup>H]thymidine were administered as described by Smith and Levine (21). Symbols: ●, 18 (*c2ts*); ○, 18 (16<sup>-</sup>*ts c2ts*).

TABLE 2. Evidence for defective particle formation after thermal induction of lysogenic strain 18 (*16<sup>-ts</sup> c2ts*)<sup>a</sup>

Lysogen	Active phage/ml	OD <sub>260</sub> /cm	Active phage/OD <sub>260</sub>
18 ( <i>c2ts</i> )	$2.4 \times 10^{11}$	3.8	$6.3 \times 10^{10}$
18 ( <i>16<sup>-ts</sup> c2ts</i> )	$1.1 \times 10^{10}$	5.6	$2.0 \times 10^9$

<sup>a</sup> OD<sub>260</sub> wavelength and active phage per milliliter were measured on CsCl purified lysates from thermally induced lysogens. The ratio, active phage/OD<sub>260</sub>, gives a measure of the viability of the phage suspensions.

markedly reduced; (ii) normal numbers of particles are produced, but most of these are noninfectious.

The total yield of particles can be assayed by determining the optical density at 260 nm (OD<sub>260</sub>) of a purified phage preparation (20). The phage lysates obtained from the thermal induction experiment of Fig. 1 were concentrated by centrifugation to equilibrium in CsCl. Both lysates gave visible bands in similar positions, suggesting that particles of similar densities are formed in both inductions. The OD<sub>260</sub> for the band from the mutant lysate is one and a half times greater than the value obtained for the control (Table 2, column 3). Thus, total particle production is not reduced on induction of *16<sup>-ts</sup>* lysogens. However, for an equal concentration of particles as determined by OD<sub>260</sub>, the infectious yield from the mutant lysogen is reduced by a factor of 30 when assayed at 25°C (Table 2). Therefore, the induced *16<sup>-ts</sup>* lysate consists largely of particles which are noninfectious even when assayed at the permissive temperature.

These results clearly show that gene *16* product is not required for P22 particle formation on induction. It might be suspected that the noninfectious particles produced after induction of *16<sup>-ts</sup>* lysogens at high temperature are probably either missing *P16* or contain inactive *P16*. The lesion in these particles is examined in a later section. The events on induction are in marked contrast to infection with P22 *16<sup>-ts</sup>*. As will be shown in the accompanying paper (9), no phage functions are detected in nonpermissive infections. Neither phage DNA, proteins, nor particles are synthesized.

The term *defective particles* will be used to refer to noninfectious particles produced after infection or induction with gene *16* mutant phage under nonpermissive conditions. When the genotype of these particles is given, it will precede this term. The manner by which the particles were produced, either infection or induction, will follow in parentheses. Thus, defec-

tive particles formed by induction of *16<sup>-ts</sup>* lysogens at the nonpermissive temperature will be designated *16<sup>-ts</sup>* defective particles (induction).

**Infective center assay for defective particles.** A simple assay for defective particles based on a complementation test was developed. Mixed infections were carried out at 25°C in *su<sup>-</sup>* strain 18 so that all cells were infected with P22 *12-*amc2** (MOI = 5), and no cell was infected by more than one *16<sup>-ts</sup>* defective particle (induction, MOI = 0.5). If complementation occurs for the function missing from the defective particle, the number of infective centers, using *su<sup>-</sup>* strain 18 as the indicator bacteria, should correspond to the number of cells which are mixedly infected. An infective center is an infected cell which yields phage that form a plaque under specific plating conditions. In this assay, infective centers should equal the number of defective particles in the suspension. The infective centers from single infection using only the thermally induced *16<sup>-ts</sup>* suspension will be equal to the number of active phage in the suspension.

P22 *16<sup>-ts</sup>* defective particles (induction) can indeed be complemented (Table 3). Mixed infections show significant increases in infective centers when compared to single infections: infection 3 shows a 23-fold increase compared to infection 1. Additional evidence that the defective particles are complemented for phage production is that the burst size for mixedly infected cells (from infection 3) is high, 154, with both amber and temperature-sensitive phage present in the yield.

The term *total particles* will be used to refer to the titer obtained with the infective center assay described above. Any difference between infective centers in single infection (active phage) and infective centers in mixed infection (total particles) is ascribed to the presence of defective particles.

TABLE 3. Infective center assay for *16<sup>-ts</sup>*-defective particles (induction)<sup>a</sup>

Phage	MOI <sup>b</sup>	Infective centers <sup>c</sup>
1. <i>16<sup>-ts</sup></i> -defective particles	0.05	$3.4 \times 10^5$
2. <i>12-<i>amc2</i></i>	5	<10 <sup>4</sup>
3. <i>16<sup>-ts</sup></i> -defective particles × <i>12-<i>amc2</i></i>	0.05:5	$7.8 \times 10^6$
4. <i>16<sup>-ts</sup></i> -defective particles	0.005	$3.2 \times 10^4$
5. <i>16<sup>-ts</sup></i> -defective particles × <i>12-<i>amc2</i></i>	0.005:5	$9.0 \times 10^5$

<sup>a</sup>  $1.6 \times 10^8$  cells were infected at 25°C.

<sup>b</sup> It was possible to estimate the MOI of defective particles by using OD<sub>260</sub>/cm to determine the concentration of particles.

<sup>c</sup> Infective centers were plated on *su<sup>-</sup>* strain 18 at 25°C.

The following observations provide additional evidence that the number of infective centers in the previously described mixed infection is a valid assay for defective particles. (i) There is a direct linear relationship between infective centers and defective particle multiplicity as long as each cell is infected with no more than one defective particle (Table 3, compare infections 3 and 5). Dilution of the defective particle suspension by a factor of 10 results in a 10-fold decrease in infective centers. (ii) In the induced *16<sup>-</sup>ts* lysate, the titer of defective particles determined by the infective center assay ( $4.2 \times 10^{11}$  per ml) agrees with the expected number calculated by OD ( $3.5 \times 10^{11}$  per ml). (iii) Both the infective center assay and OD readings give similar ratios of total particles to active phage (28 by the infective center assay and 31 by OD<sub>260</sub>) for the lysate from thermal induction of *16<sup>-</sup>ts* lysogens. (iv) Lysates obtained from thermal induction of *16<sup>+</sup>* lysogens show no difference in active phage per milliliter and total particles per milliliter using the infective center assay. This is the expected result if there are no defective particles in the phage suspension.

The *16<sup>-</sup>am* defective particles (infection) described by Botstein et al. (6) and King et al. (13) can also be detected using the infective center assay, as shown in Table 4.

**Temperature shift-up experiment.** If *P16* is a virion protein necessary early in the infective process, shifting a P22 *16<sup>-</sup>ts*-infected culture from the permissive to the nonpermissive temperature after completion of the early step requiring *P16* should result in a situation analogous to induction, expression of early and late phage genes, and particle production. The particles produced should be defective just as they are after induction of a *16<sup>-</sup>ts* lysogen at 41 C. This type of experiment was carried out as described below.

TABLE 4. Infective center assay for *16<sup>-</sup>am*-defective particles (infection)<sup>a</sup>

Infection	Infective centers
1. <i>16<sup>-</sup>amN121c1</i> -defective particles.	$7.0 \times 10^2$
2. <i>16<sup>-</sup>amN121c1</i> -defective particles × <i>18<sup>-</sup>ts1</i> .....	$4.3 \times 10^6$
3. <i>16<sup>-</sup>amH60c1</i> -defective particles..	$1.0 \times 10^3$
4. <i>16<sup>-</sup>amH60c1</i> -defective particles × <i>18<sup>-</sup>ts1</i> .....	$1.7 \times 10^7$
5. <i>18<sup>-</sup>ts1</i> .....	$<10^5$

<sup>a</sup> The infective center assay was adapted to test for *16am*-defective particles.  $2 \times 10^6$  *su<sup>+</sup>* cells were infected at 25 C. Infective centers were assayed using *su<sup>+</sup>* indicator at 41 C.

Sensitive cells were infected at 25 C with P22 *16<sup>-</sup>ts2* (MOI = 5). After adsorption, free phage were inactivated by antiserum and the culture was diluted  $10^4$ -fold. At intervals, three samples were removed. One sample was plated at 25 C for a single-step growth curve, the second was immediately shaken with chloroform and assayed for intracellular phage at 25 C, and the third was shifted to 41 C, incubated for a total of 180 min, and then shaken with chloroform and assayed at 25 C for both active phage and total particles.

Figure 2 presents the results of the temperature-shift experiment. The latent period at 25 C is about 80 min and intracellular phage start to accumulate at about 60 min. A 10-min incubation at 25 C, followed by a shift to 41 C for the remaining time, is sufficient to give a total particle yield exceeding that obtained in the permissive infection. (The total particle yields for samples shifted to 41 C prior to 100 min are always slightly larger than the yield in the permissive infection.) These results demonstrate that P22 *16<sup>-</sup>ts* infections at 41 C are blocked very early. However, in agreement

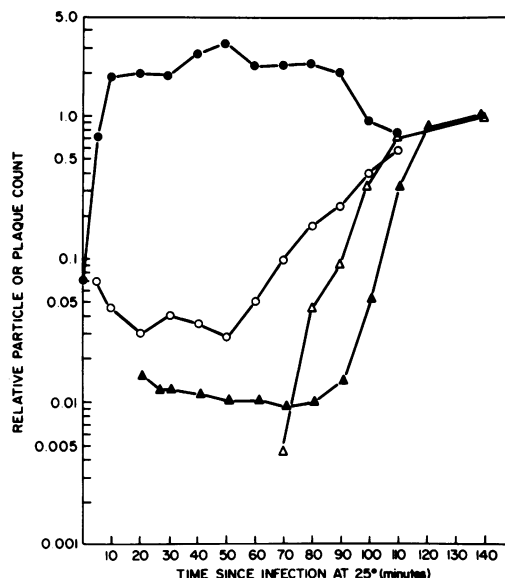


FIG. 2. Temperature-shift experiment. The infection was initiated at 25 C with P22 *16<sup>-</sup>ts2* (MOI = 5). At intervals, three samples were removed. One was plated for a single-step growth curve (▲), the second for intracellular phage (△), and the third was shifted to 41 C, incubated for a total of 180 min, chloroformed, and assayed for total particles (●) and active phage (○). All numbers are relative to the active phage titer in the lysate from the permissive infection.

with our prediction, mostly defective particles are produced when P22 *16<sup>-ts</sup>*-infected cells are shifted to the nonpermissive temperature after the early step has occurred (10 min).

At the permissive temperature, no defective particles are formed. Total particles are always equal to active phage. However, active phage production in samples shifted-up prior to 50 min is substantially reduced compared to the permissive infection. Incubation at 25 C for longer than 50 min results in an increase in active phage, which is correlated with the rise in intracellular phage. Normal yields of active phage are not obtained until samples are incubated at 25 C for at least 90 min. These results show that there is a second step late in the infection which, when blocked, prevents the formation of active phage.

Although an early shift-up gives a reduced number of active phage compared to a permissive infection, this active phage yield is still 10 times greater than that from a completely nonpermissive infection. This early increase in active phage is caused by leakiness for the late step. A similar fraction of the total particles from induction of *16<sup>-ts</sup>* lysogens at 41 C is also active phage.

Thus, temperature shift experiments show that gene *16* product is required both early and late during infection for the production of active phage. If the gene *16* protein is rendered inactive early, then few progeny particles are detected. If the gene *16* protein is inactivated between 10 and 50 min after infection, the early step requiring gene *16* protein has occurred and a normal number of particles are produced. However, most of these particles are defective. A shift-up immediately after the early step requiring gene *16* product creates a situation analogous to induction of *16<sup>-ts</sup>* lysogens at 41 C; these infected cells lyse and release large numbers of defective particles.

**Comparison of the protein composition of defective particles and phage using acrylamide gel electrophoresis.** Botstein et al. (6) have shown that mature P22 phage particles contain seven different proteins called, in order of decreasing molecular weight, P1, P9, P16, P5 (the major protein species), P20, P26, and PX. Noninfectious particles produced by a nonpermissive P22 *16<sup>-am</sup>* infection are missing P16. This was demonstrated by disrupting [<sup>14</sup>C]amino acid-labeled phage or particles, separating the proteins by electrophoresis in a high-resolution discontinuous SDS-polyacrylamide gel system, and analyzing the gels by autoradiography.

To determine if *16<sup>-ts</sup>* defective particles (in-

duction and shift-up infection) are also missing P16, similar experiments were carried out. Defective particles and phage, purified in CsCl, were disrupted in SDS, and the protein components were analyzed using a high-resolution discontinuous SDS-polyacrylamide gel system (19). Coomassie blue stain was used to detect the protein bands. Using proteins with known molecular weight, the bands were correlated with the virion proteins identified by Botstein et al. (6). The Coomassie blue banding pattern for P22 *c2* revealed five different proteins: P1, P9, P16, P5, and P20 (Fig. 3). The most likely explanation for the failure to detect P26 and PX is that these proteins are very minor components of the virion and their concentration on the gels is too low to pick up detectable stain. Both *16<sup>-am</sup>*-defective particles (infection) and *16<sup>-ts</sup>*-defective particles (shift-up infection) are missing P16 (Fig. 3). This protein is found in wild-type phage and in both P22 *16<sup>-ts</sup>* and P22 *16<sup>-am</sup>* produced under permissive conditions. P22 *16<sup>-ts</sup>*-defective particles (induction) are missing P16 and P9, the tail protein (Fig. 4). The absence of P9 is expected since phage from induction of wild-type lysogens are deficient in tails (10). These observations agree with those of Botstein et al. (6) and clearly show that *16<sup>-am</sup>*- and *16<sup>-ts</sup>*-defective particles, whether from infection or induction, are missing the same protein.

The defective particles have a slightly higher density than infectious phage in CsCl, which is not due to any detectable change in the sedimentation coefficient or density of the DNA (data not shown). This increase in density is expected for particles having an increased DNA to protein ratio.

**P22 *16<sup>-ts</sup>* virions contain heat-labile gene 16 protein.** P22 *16<sup>-ts</sup>* virions contain P16 (Fig. 3), yet infections at the nonpermissive temperature are blocked early. We have previously suggested that P16 in this mutant phage is thermolabile. The following experiments demonstrate that, although the P22 *16<sup>-ts</sup>c2* virion is no more sensitive to high temperatures than P22 *16<sup>+</sup>* virions, the P16 contained in this mutant virion is irreversibly denatured after infection at the nonpermissive temperature.

A comparison of the heat sensitivities of P22 *16<sup>-ts</sup>c2* and P22 *16<sup>+</sup>c2* was undertaken. Both phages are not affected by exposure to temperatures as high as 52 C for 1 h. At 65 C, P22 *16<sup>-ts</sup>c2* virions are at least as stable to heat as P22 *16<sup>+</sup>c2* virions and may be even more resistant (Fig. 5).

Although the P22 *16<sup>-ts</sup>* virion is not differentially sensitive to high temperatures, its P16

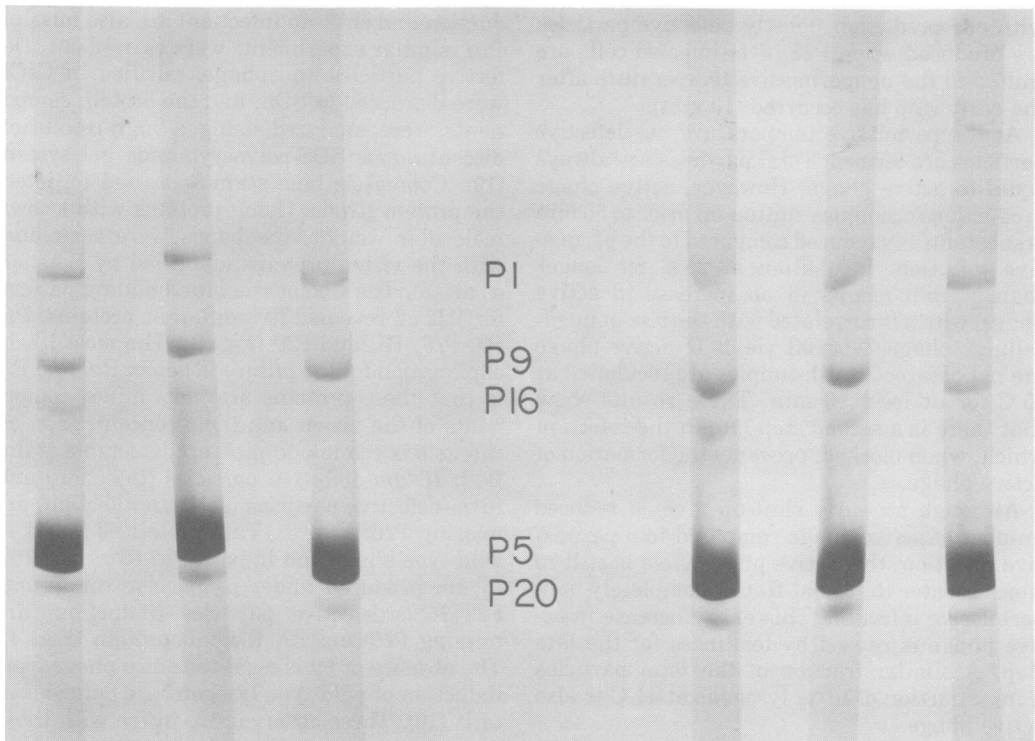


FIG. 3. Protein composition of phage and defective particles from infection, resolved by SDS-acrylamide gel electrophoresis. For the first three gels, samples are from left to right: P22 *c2*, P22 *16<sup>-amc1</sup>* phage (from a permissive infection), and *16<sup>-am</sup>*-defective particles (infection). For the second three gels, the phage used are, from left to right: P22 *c2*, P22 *16<sup>-tsc2</sup>* phage (from a permissive infection), and *16<sup>-ts</sup>*-defective particles (shift-up infection). The protein bands have been identified, from top to bottom as: P1, P9, P16, P5, and P20 (6).

may be thermolabile after infection at 41 C. This would explain the early block in the nonpermissive infection. In the absence of functional P16, the host cell survives (9). If P16 is irreversibly inactivated after infection with P22 *16<sup>-ts</sup>* at 41 C, then infected cells should neither be killed nor produce phage after a subsequent shift-down to 25 C.

Cells were infected with P22 *16<sup>-tsc2</sup>* at 41 C, and at various times samples were plated at 25 C for plaque-forming ability (single step growth curve) and cell survivors. Only 10% of the infected cells produced plaques after 12 min at 41 C (Fig. 6A). The number of plaques continues to fall until 35 min and then starts to increase. The late increase in plaques is probably due to leakiness of the *16<sup>-ts</sup>* mutation, resulting in the formation of a few active phage. As the number of plaques decreases, survival of the host cells increases. Five minutes after infection at 41 C, fewer than 50% of the cells form colonies when plated at 25 C (Fig. 6B). However, after 12 to 15 min at 41 C, most of the infected cells survive on shifting to the permissive temperature. These observations are evi-

dence for the irreversible inactivation of P22 *16<sup>-ts</sup>* infections at the nonpermissive temperature; however, as discussed below, heat lability of P16 is not the only possible explanation for this observation.

It is tempting to conclude that P22 *16<sup>-ts</sup>* infections at 41 C are inactivated because the P16 component of the virion is irreversibly heat labile. Alternatively, in the absence of functional P16, the phage genome, or another phage component, may be secondarily rendered irreversibly inactive. Since complementation for the early step requiring P16 occurs in a simultaneous mixed infection, it is possible that superinfecting with P16<sup>+</sup> phage can also complement and, therefore, rescue a primary P22 *16<sup>-ts</sup>* infection. Rescue would not be expected if a secondary inactivation had occurred. However, if the only cause for irreversible inactivation of the P22 *16<sup>-ts</sup>* infection is heat denaturation of P16, then the infection might be rescued.

The following infections were carried out to determine whether superinfecting P22 *20<sup>-amc2</sup>* can complement P22 *16<sup>-tsc2</sup>*. *Su<sup>-</sup>* strain 18 was infected at 41 C with P22 *16<sup>-tsc2</sup>* (MOI = 0.1).

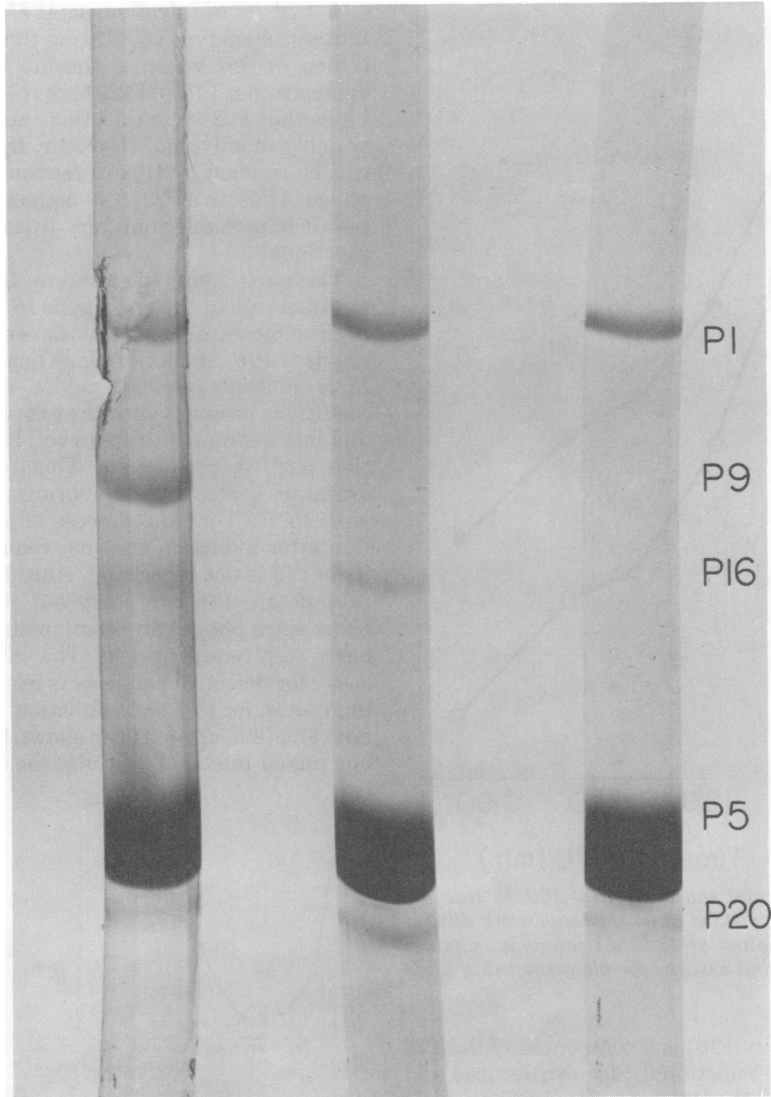


FIG. 4. Protein composition of phage and defective particles from induction, resolved by SDS acrylamide gel electrophoresis. Samples used are from left to right: P22  $c2$  (from infection, as a control), P22  $c2ts$  (induction), and  $16^{-ts2ts}$  defective particles (induction). The bands can be matched with those from Fig. 3.

Ten minutes later an aliquot was diluted and assayed for infective centers. At the same time, a second aliquot was superinfected with P22  $20^{-amc2}$  (MOI = 10). In addition, an aliquot of uninfected cells was infected with P22  $20^{-amc2}$ . After adsorption, these last two samples were assayed for infective centers. Only 12% of the P22  $16^{-ts}$ -infected cells form infective centers (Table 5, infection 1). No infective centers are detected for the P22  $20^{-amc2}$  infection (Table 5, infection 2). However, in the sequential infection, all mixedly infected cells produce infective centers (Table 5, infection 3).

Superinfecting P22  $20^{-amc2}$  can complement and, therefore, rescue a P22  $16^{-ts2}$  primary infection at 41 C. It can be concluded that a secondary irreversible inactivation, such as damage to the phage genome, does not take place in the absence of functional P16. Therefore, P22  $16^{-ts2}$  infections at 41 C are abortive, probably because P16 is irreversibly denatured.

#### DISCUSSION

Gene 16 product, a head protein, is required both early and late in infection. The



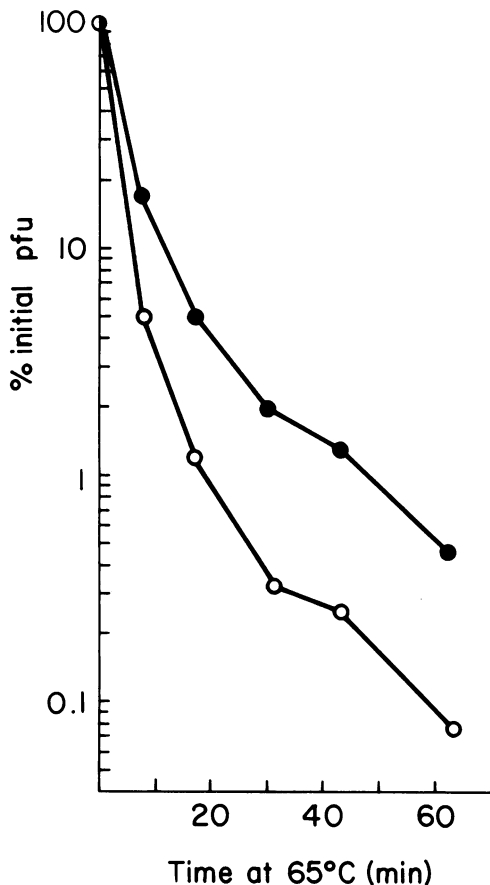


FIG. 5. Thermal inactivation of P22 16-tsc2 (●) and P22 c2 (○) virions at 65°C. Phage were diluted into buffered saline at 65°C. At intervals, samples were removed and assayed for plaque-forming units (PFU) at 25°C.

gene 16 protein (P16) is a component of the P22 virion (6, 13). Functional P16, synthesized and incorporated into particles in the previous cycle of phage production, has a necessary early function for production of virus in the subsequent infection. Temperature-shift experiments with P22 16-ts demonstrate the early and late requirement for P16. If P16 is rendered inactive early, no particles are produced. If P16 is inactivated between 10 and 50 min after infection, the early step has occurred and particles are formed. However, most of these particles are defective since they lack the gene 16 protein.

It has been shown by SDS-polyacrylamide gel electrophoresis that defective particles missing P16 are produced after: (i) an early (10 to 50 min) temperature shift-up infection with P22 16-ts; (ii) a nonpermissive infection with P22 16-am; and (iii) induction at the nonpermissive temperature of lysogens containing 16-ts prophage.

A P22 16-ts infection at 41°C has an early mutant phenotype suggesting that the P16 contained in this virion is thermolabile. Further evidence that P16 in P22 16-ts is heat denaturable is that P22 16-ts infections at 41°C are irreversibly inactivated. However, these infections can be rescued by superinfection with P22 16+ phage. Thus, in a P22 16-ts nonpermissive infection it is probable that very little of the P16 is functional.

**The early gene 16 function.** Several important facts about the early gene 16 function have been demonstrated in this paper. (i) Infection requires P16, induction does not. Induction of 16-ts mutant lysogens under nonpermissive conditions proceeds with the expression of early and late genes, resulting in cell lysis and defective particle production. This response is in dramatic contrast to nonpermissive infections with 16-ts. That the process of phage production after induction does not require the activity of P16 is not surprising, since P16 is a virion protein. (ii) P16 can carry out its function in trans since phage can be complemented for the early step requiring P16. The infective center assay for defective particles is based on complementation for this early function. (iii) Not only has complementation been shown for simultaneous mixed infection, but also for sequential in-

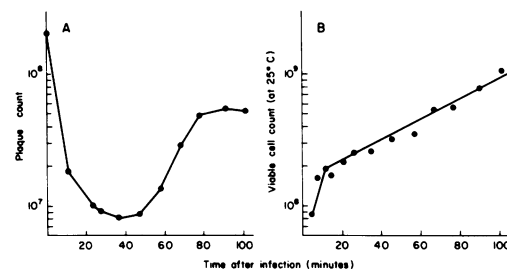


FIG. 6. Irreversible inactivation of P22 16-tsc2 infections at 41°C. Strain 18, at a concentration of  $2 \times 10^8$  cells/ml, was infected at 41°C with P22 16-tsc2 (MOI = 5). Various times after infection, samples were assayed for plaques (A) and colony formers (B) at 25°C.

TABLE 5. Rescue of a P22 16-tsc2 infection at 41°C by superinfecting P22 20-amc2<sup>a</sup>

Infection	Superinfection	Infective centers (Su <sup>-</sup> , 25°C)
1. 16-tsc2		$2.4 \times 10^6$
2. 20-amc2		$10^5$
3. 16-tsc2	20-amc2	$2.1 \times 10^7$

<sup>a</sup> Su<sup>-</sup> strain 18, at a concentration of about  $2 \times 10^8$  cells/ml, was infected at 41°C with P22 16-tsc2 (MOI = 0.1), so that  $1.9 \times 10^7$  cells/ml were infected with this phage.

fections. *P16<sup>-</sup>* primary infections can be complemented, and the gene *16<sup>-</sup>* genome rescued, by superinfecting with *P16<sup>+</sup>* phage. Thus, in the absence of *P16*, the infection is blocked at some point in the normal sequence of events, but the phage genome is not irreversibly damaged. That is, in the absence of *P16* the phage DNA is not susceptible to host-induced damage, thereby eliminating a protecting role for *P16*.

**Gene 16 function is not an N type function.** Bezdek and Soska (1) claimed that the gene *16* product has a basic regulatory function similar to the N function of phage  $\lambda$  (8). The temperature-sensitive mutant which they describe, P22 *X<sup>-ts</sup>*, is identical with P22 *16<sup>-tsc2</sup>*, the phage used in this report. All their observations agree with those presented in this report and the accompanying paper, except that they did not detect lysis after UV induction of LT2 (*X<sup>-tsc<sup>+</sup></sup>*) at 39 C. Failure to make this observation, plus the absence of active phage production after induction, led them to conclude that gene *16* product is necessary for the expression of any phage genes on induction as well as on infection.

In this paper, we report that thermal induction of 18 (*16<sup>-ts</sup> c2ts*) at 41 C results in cell lysis and defective particle production. We have also observed that lysis follows UV induction at high temperature of 18 (*16<sup>-tsc<sup>+</sup></sup>*), and mitomycin C induction of 18 (*16<sup>-tsc<sup>+</sup></sup>*) at the nonpermissive temperature results in high yields of defective particles (data not shown). These observations show that late functions are expressed in the absence of functional *P16* after induction.

Bezdek and Soska (2) carried out a temperature-shift experiment, keeping P22 *X<sup>-ts</sup>*-infected cells at permissive temperature for varying periods of time before shifting to nonpermissive conditions. Since they found only low levels of active phage when cells were maintained at low temperature for considerable times before being shifted to high temperature, they concluded that the gene *X* product (now the gene *16* product) is required throughout the entire infection in order that a normal burst of active phages be obtained and that the entire lytic cycle of phage P22 growth is under the positive control of gene *X*.

Using the infective center assay for the detection of defective particles, we have demonstrated in a similar temperature-shift experiment that there are two separate events in P22 infections requiring the product of gene *16*: an early step necessary for any particle production at all and a late step required for the particles to become infectious. If the early step occurs but the late step is prohibited by an appropriate temperature shift of P22 *16<sup>-ts</sup>*-infected cultures,

normal yields of predominantly defective particles are produced. The yields can be assayed by the infective center assay. The defective particles are missing the gene *16* protein. Thus, a shiftup of P22 *16<sup>-ts</sup>*-infected cells between 10 and 50 min after infection results in low infectious phage yields because the gene *16* product fails to be incorporated into the virion, and not because, as suggested by Bezdek and Soska, this gene product is required for positive control of early and late phage functions.

**The role of P16 in morphogenesis.** *P16* is an essential component of the P22 virion; it must be incorporated into the particle in the productive response after either infection or induction for the progeny to be infectious. King et al. (13) have shown that *P16* is present in the 240S prohead particles, which are early intermediates in the head morphogenesis pathway. The absence of *P16* has no effect on the subsequent assembly process. Without *P16*, phage DNA is packaged into particles which are morphologically indistinguishable from wild-type virions when examined by electron microscopy (6). The DNA contained in these particles is normal when both density and sedimentation properties are examined (Hoffman, unpublished data). Therefore, *P16* is not involved in packaging the DNA. These noninfectious particles cannot be activated in vitro (6; Hoffman, unpublished data). However, as we have shown, they can be complemented in mixed infections, and, therefore, can be activated in vivo.

Approximately one in thirty *16<sup>-ts</sup>*-defective particles (produced by induction and shift-up infection) is infectious and, therefore, probably contains *P16*. The leakiness for the late step with this mutant can be explained in the following manner. It is possible that *P16* is synthesized at 41 C, but is not incorporated into most virions because it is denatured. This denaturation effect is not absolute, allowing *P16* to be incorporated into some particles.

Noninfectious particles lacking P20 are produced in a P22 *20<sup>-am</sup>* nonpermissive infection (6). These particles appear normal by electron microscopy examination (6). Thus, it appears that P20, like *P16*, is a virion protein which has no direct role in particle assembly, but is necessary for infectivity. P20<sup>-</sup> particles cannot be activated in vitro (6) and are only poorly complemented in mixed infections (Hoffman, unpublished data).

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