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Head Morphologies in Bacteriophage T4 Head and Internal Protein Mutant Infections

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Escherichia coli infected with phage T4 mutants defective in synthesis of the three major internal proteins found in the phage head, IPI$^-$, IPII$^-$, IPIII$^-$, or IP° (lacking all three) were examined in the electron microscope for head formation. Infection with IPI$^-$ or IPII$^-$ does not appear to induce increased aberrant head formation, whereas IPIII$^-$ or IP° infections result in production of polyheads and viable phage. Multiple mutants of the early head formation genes 20, 21, 22, 23, 24, 31, 40 and IP° were constructed. Combination with IP° increases polyhead formation when head formation is not blocked at a more defective stage but results in a qualitative shift to lump formation in association with gene 22 mutants. Thin-sectioning studies show morphologically similar cores in amber 21 and 21am IP° τ particles. These morphological observations, genetic evidence for interaction between ts mutants in gene 22 and the IP mutants, and analysis of the protein composition of τ particles further support the idea that p22 and the internal proteins form an unstable assembly core necessary for an early stage of head formation (M. K. Showe and L. W. Black, 1973).

Seven bacteriophage T4 head genes are known to determine the overall morphology of the capsid. A defect in any of these form-determining genes (Y genes), or genes 20, 21, 22, 23, 24, 31, and 40, results in production within the infected cell of aberrant head structures-lumps, single- or multilayered polyheads, and τ particles (9, 14). The types of structures associated with defects in each of the Y genes established their general functions in head shape determination; studies with multiple Y gene mutants established dominance relations among them in form determination (19). The ultrastructure of several of the aberrant heads has been intensively investigated (1, 14, 25). Biochemical studies show that aberrant head structures are composed primarily of the protein product of gene 23, designated p23. The products of a number of the other Y genes have also been identified and some are found in the capsid or in aberrant structures (18, 20, 22). The products of other Y genes do not, however, appear in the completed head (22). A number of the precursor head proteins are cleaved in vivo only when the normal capsid structure is assembled, while the aberrant structures are formed of the uncleaved head precursor proteins (8, 10, 15, 16).

A number of proteins found in the normal T4 head proved not to be products of any known T4 head genes. Among these are the three major internal proteins found inside the phage head (2, 6, 11, 24). Isolation of mutants which prevented synthesis of each of these internal proteins allowed some inferences about their functions. Because the internal proteins are dispensible, they are not required for DNA condensation or capsid formation, although in the absence of one of them, IPIII, head assembly is partially defective (4, 22). All the internal proteins can be isolated in a complex with p22 and can be found along with p22 in defective 23ts heads, τ particles, and polyheads (18, 20, 22). These observations and the phenotypes of mutants defective in synthesis of p22 and internal proteins suggested that these proteins were involved in initiating capsid assembly and constituted the core material seen in earlier morphological studies of defective head structures (22).

In view of the localization of internal proteins in the cores of early head precursors, and the effects of internal protein mutation on phage yield and head protein conversion, it was of interest to determine the effects of the internal protein mutations, singly and in combination, upon head formation in infected cells. We wished to determine how these mutations inter-

acted with the originally identified T4 head gene mutations in selecting among possible defective head structures in head mutant-internal protein mutant combinations. We also wished to establish the presence of the internal proteins in defective morphological structures, the possible effect of an absence of internal proteins upon the morphology of the cores within certain aberrant head forms, and the morphological phenotype associated with a complete absence of core proteins.

MATERIALS AND METHODS

(i) Bacteria and bacteriophage. *Escherichia coli* B^E and S/6 were nonpermissive strains for amber mutants. Amber mutant stocks were grown in *E. coli* CR63, which is permissive for amber mutants. The isogenic *E. coli* strains $B40su_I^+$ and P301 pm^- were used to determine the influence upon burst size of suppression of amber (am) mutants in temperature-sensitive (ts) amber (ts-am) multiple mutants (Gorini, personal communication).

Amber and temperature-sensitive mutants of phage T4D were from R. H. Epstein and W. B. Wood. Isolation of internal protein mutants of phage $T4D^+$ and preparation of multiple mutants of the internal proteins and of am and ts mutants in T4 head genes were as described (4). Bacteriophage T4 mutants prepared and used in this study are listed in Table 1.

(ii) Media and growth of bacteria. Liquid cultures were grown in M9s medium (0.7% Na_2HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 0.4% glucose, 10^{-3} M $MgSO_4$, 10^{-4} M $CaCl_2$, 0.25% Casamino Acids [charcoal treated]). Radioactive labeling was carried out in the above medium without Casamino Acids (M9 medium). Phage-dilution buffer was 0.026 M Na_2HPO_4, 0.069 M NaCl, 0.022 M KH_2PO_4, 10^{-3} M $MgSO_4$.

Infected bacteria: *E. coli* B^E were grown at 37 C to 2×10^8 to 4×10^8 cells/ml and infected with a multiplicity of four and superinfected with the same multiplicity 7 min later to insure lysis inhibition. In the case of ts mutants, 41 C was the nonpermissive temperature.

(iii) Electron microscopy. Single lysed cells were examined by in situ lysis as described by Kellenberger et al. (14). Thin sections of bacteria were fixed in glutaraldehyde-uranylacetate according to Séchaud and Kellenberger (21). Negative staining was with 2% phosphotungstic acid neutralized to pH 7.0. Samples were examined in a Siemens Elmiskop 101 electron microscope.

(iv) Protein composition and purification of defective heads. Defective heads (23ts heads or τ particles) were purified from bacteria infected with tsL65 or tsA78 at 42 C (for 23ts heads) or with gene 21 (amN90) at 37 C (for τ particles). After labeling with a ^{14}C-labeled amino acid mixture (see ii), the bacteria were centrifuged from suspension in 10 ml of M9 at 35 (42 C) or 45 (37 C) min. The bacteria were suspended in spheroplasting mixture (10 λ of 2.7 mg of EDTA/ml, 20 λ of 10 mg of egg-white lysozyme/ml) at 0 C for 10 min. Then 60 λ of 0.1 M $MgSO_4$, 30 λ of RNase (1 mg/ml), and 40 λ of DNase (1 mg/ml) were added. After 2 min at 37 C, the suspension was frozen, thawed, and incubated at 37 C for 5 min. The suspension was chilled to 0 C, and 0.4 ml of phage-dilution buffer and Nonidet to 1% were added. Following incubation at 37 C for 5 min, the suspension was chilled and centrifuged 15 min at 5,000 rpm, and the supernatant was centrifuged on a CsCl step gradient (13) for protein separation (lowest density layer, 1.35 g/ml) in an SW50 rotor for 22 min at 44,000 rpm. The lowest sharp visible band at the bottom of the gradient contained defective heads and was removed, dialyzed against water, and lyophilized. Discontinuous sodium dodecyl sulfate-acrylamide gel electrophoresis (16) with a urea gradient (C. Castillo, manuscript in preparation) and radio-immunodiffusion (6) were as described.

TABLE 1. *Phage T4 mutants*

T4 mutants	Mutant IP°[a]
20 (amN50)	20 (amN50) IP°
21 (amN90)	21 (amN90) IP°
22 (amB270)	22 (amB270) IP°
23 (amH11)	23 (amH11) IP°
24 (amN65)	24 (amN65) IP°
31 (amN111)	31 (amN111) IP°
40 (tsL177)	40 (tsL177) IP°
40 (tsL84)	40 (tsL84) IP°
22 (tsL147)	22 (tsL147) IP°
	22 (tsL147) IPI^- (HA35)
	22 (tsL147) $IPII^-$ (HA100)
	22 (tsL147) $IPIII^-$ (HA9)
22 (tsA74)	22 (tsA74) IP°
17 (tsL2)	17 (tsL2) IP°
20 (tsA3)	20 (tsA3) IP°
21 (tsN8)	21 (tsN8) IP°
23 (tsA78)	23 (tsA78) IP°
23 (tsL65)	23 (tsL65) IP°
24 (tsN29)	24 (tsN29) IP°
31 (tsA70)	31 (tsA70) IP°
41 (tsA14)	41 (tsA14) IP°
49 (tsC9)	49 (tsC9) IP°
55 (tsA81)	55 (tsA81) IP°

[a] IP° = IPI^- (HA35), $IPII^-$ (HA100), and $IPIII^-$ (HA9).

RESULTS

(i) Head morphologies of IP^- mutants. Phage T4 mutants have been isolated which prevent synthesis of functional internal proteins. Since these chain termination mutations in the structural genes for the internal proteins grow on nonpermissive bacteria, and since deletions of the internal protein genes are also viable, the internal proteins must be non-essential. IPI appears to be required to initiate infection of certain hosts (4, 5, 22).

When single cells infected with mutants of IPI (Fig. 1a), IPII (Fig. 1b), and IPI, IPII (Fig. 1c) are examined by the in situ lysis technique

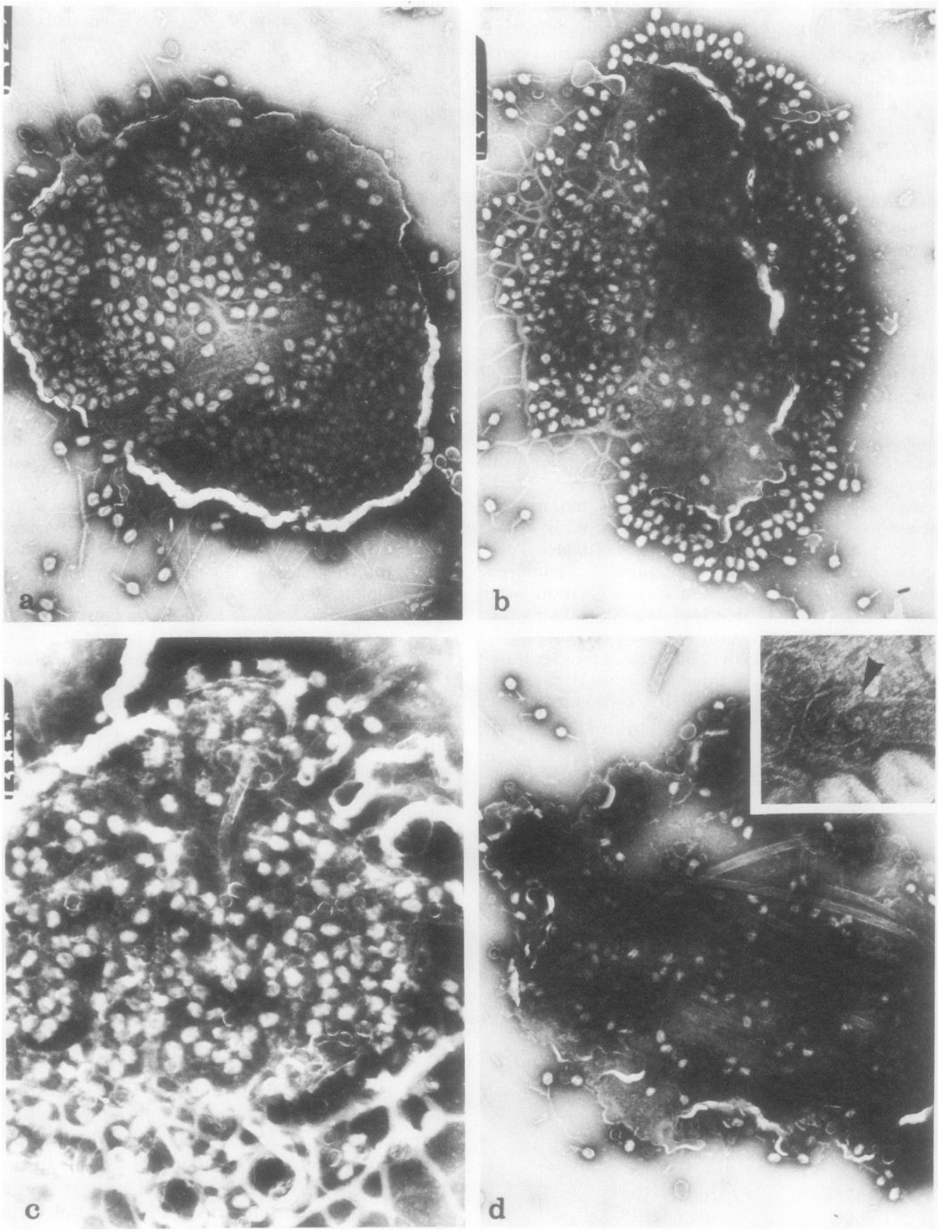

FIG. 1. *Micrographs of in situ lysed E. coli B^s cells infected with internal protein mutants. Cells were lysed in situ with 0.01% OsO_4 after infection (1a) 90 min after infection with IPI (HA35); (1b) 90 min after infection with IPII (HA100); (1c) 60 min after infection with IPI, IPII (HA35, HA100); (1d) 90 min after infection with IPIII (HA9). The insert to Fig. 1d shows the end of a capped polyhead (arrow). Note the presence of large numbers of free tails only in the IPIII$^-$ infection. Preparations were negatively stained with 2% phosphotungstic acid.* ×23,000.

late after infection, cells are seen to be filled with apparently normal phage particles, the great majority of which are apparently filled with DNA. The small number of polyheads which are also present in the IPI- and IPII-infected cells are also characteristic of wild-type T4 infection at the same time (90 min after infection). Therefore by electron microscopy observation the absence of the two small basic internal proteins, IPI and IPII, appears to have very little if any effect upon normal morphological development of mature phage particles. The same conclusion was also drawn from pulse-chase experiments with IPI and IPII mutant infections of *E. coli* B and measurements of phage yield. In such infections the cleavage of head precursor proteins and the rate and efficiency of phage formation appear indistinguishable from a wild-type infection (unpublished observations). In contrast, head assembly in IPIII$^-$ or IP° infections is defective as measured by lowered phage yield and less complete cleavage of head precursor proteins (4, 22). Mutants in the gene for IPIII also produce a considerably smaller number of phage particles and large numbers of polyheads when single infected cells are examined at the same late time (Fig. 1d). When a triple mutant defective in synthesis of all three internal proteins (IP°) is examined, a very similar collection of phages and polyheads is seen at late times. For most of the following studies, IP° mutants have been used and, in view of our studies of the single mutants, we attribute the assembly defectiveness of this multiple mutant largely to the IPIII mutation.

When single cells infected with IP° mutants are examined early after infection (17 min at 37 C), very shortly after the first phage particle appears in a wild-type infection, a mixture of phages, polyheads, and τ particles is seen (Fig. 2). The observation that IP° morphogenesis is defective throughout infection argues against the possibility that aberrant structures result from depletion of a bacterial protein substitute for IPIII late in infection. It is apparent that IPIII mutants, like gene 24 mutants, give rise to polyheads early in infection. This is in contrast to mutants in genes 20 and 21, which also produce polyheads but only after a considerable lag period (3, 17). We have also observed that a high frequency of IP° polyheads with visible ends reveal a hemispherical cap (Fig. 1d), this apparently contrasts with polyheads produced by mutants in genes 20 and 40 which have been reported to produce 98% open-ended tubes (19). The IP°- and IPIII-infected cells also produced some multilayered polyheads, although most of the polyheads seen in the in situ lysed cells are single layered.

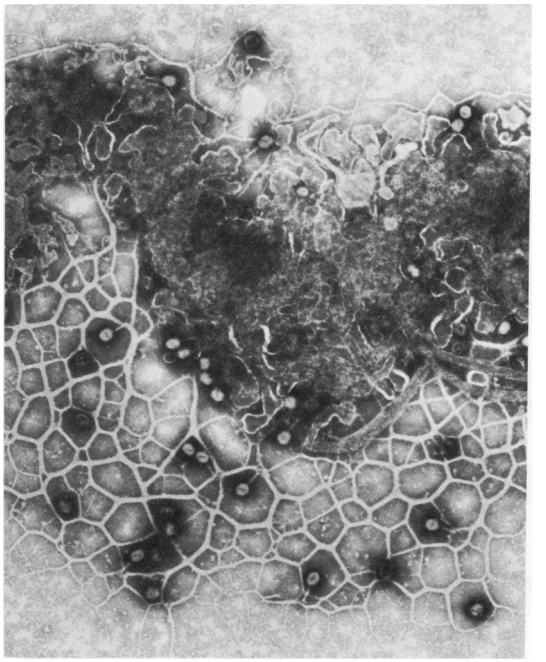

FIG. 2. *Micrograph of an in situ lysed E. coli B^E cell 17 min after infection at 37 C with IP°. ×22,000.*

(ii) Head structures produced by head gene-internal protein gene multiple mutants. A complete catalogue exists of defective head structures produced by amber and temperature-sensitive mutants in the seven T4 head shape-determining genes (Y-genes), genes 20, 21, 22, 23, 24, 31, and 40 (19). Because the IPIII$^-$ mutation depresses normal head formation, and stimulates polyhead synthesis, it was of interest to determine what structures predominated in multiple mutant-infected cells. Therefore multiple mutants of the type amYIP° were constructed, where Y is the T4 early head shape-determining gene (see Table 1). These mutants were used to infect *E. coli* B, and single cells were examined at various times after infection by the in situ lysis technique. For most of the multiple mutants, 5 to 10 lysed cells were examined at three different times after infection. These were compared with a smaller number of cells infected with the single mutant, since for the single mutants quantitation of the defective head shapes produced at various times has been reported (19). The structures produced were not carefully examined or measured for ultrastructural changes; only gross changes in structure or types of structure were determined.

In most of the multiple mutants, there is no

difference in the types of structures produced in the multiple versus the single mutant-infected cells, and our results are in agreement with observations reported earlier for the single mutant-infected cells (19). In both 23 and 31 gene-defective infections of *E. coli* there are no organized head-related forms, in the former because of the absence of the major capsid protein p23, and in the latter because of the apparent precipitation of p23 in amorphous aggregates within the cell (lumps). These forms are the same in 23am IP° and 31am IP° infections. Mutations in genes 20 and 40 give rise to polyheads in the infected cells. In cells infected with 20am IP° or 40ts IP° mutants, single-layered polyheads are still the only observable head-related structure (Fig. 3a). In cells infected with mutants in gene 21, the predominant structure produced is the τ particle (Fig. 3b). In cells infected with gene 21 IP° mutants, τ particles still appear, but a considerably larger proportion of polyheads is produced (compare Fig. 3b and c). Similarly, in 24am IP° mutants the number of polyheads relative to the number of τ particles seems to be increased relative to the ratio found for the single 24am mutant (Fig. 3d). In the 24am IP° mutant-infected cells, polyheads appear to be the predominant structure.

Quantitation of the structures produced in many 21 IP°- and 24 IP°-infected cells confirms the impression of a considerable shift to polyhead production (Table 2). In both multiple mutants, the ratio of polyheads to τ particles is considerably greater than that of the single head gene mutant examined at the same time, particularly in the case of 21 IP°.

The only apparent qualitative shift in aberrant structure formation in the head gene IP° multiple mutant series is with multiple mutant 22 IP°. Multilayered polyheads are produced by gene 22 (amB270) in the infected cell, whereas gene 22 (amB270) IP° produces no polyheads but only lumps at 40 min after infection (Fig. 4a, b). Even by 90 min after infection with gene 22 (amB270) IP°, only one-half the infected cells has produced one multilayered polyhead and in these cells lumps remain the predominant structure (Fig. 5a, b).

In 22ts L147 IP°-infected cells there seems to be less of a total shift to lump production, although many lumps, as well as multilayered polyheads, are present. In our observations, however, gene 22 tsL147 also appeared to make significantly fewer multilayered polyheads than single-layered polyheads when compared with gene 22 amB270 and is therefore apparently less defective than B270. The shift to lump production in B270 IP°- and 22 tsL147 IP°-infected cells is supported by measurements of membrane debris fraction-associated radioactivity, which is similar to that characteristic of single 31am mutant infections but not single 22am mutant infections (22). As far as could be determined, the lumps produced by the 22am or ts IP° infections appear the same as in the 31am infections.

(iii) Interactions between head and internal protein genes. Direct biochemical isolation of a protein complex (22), and morphological observations of cells infected with Y gene-IP° mutants (ii), suggest the internal proteins and p22 interact during head assembly. If the p22 IP protein complex exists and is important for head formation in vivo, a mutational change in one of its components could alter a requirement for the others. It is probable that many temperature-sensitive mutants produce gene products which differ structurally at permissive temperature from wild type and are only partially functional. A change in a protein which participates in the temperature-sensitive step could dramatically affect the ability of the temperature-sensitive protein to carry out this step even at permissive temperature; a defect in a protein with unrelated function would not be expected to interfere with completion of the temperature-sensitive step. Under this assumption, an attempt was made to identify T4 genes whose products interact with or are functionally related to the internal proteins by construction of ts-IP° multiple mutants.

Table 3 summarizes a series of burst-size measurements of various temperature-sensitive mutants and temperature-sensitive mutant-IP° multiple mutants on isogenic suppressor and nonsuppressor bacteria at permissive temperature. A large number of ts mutants were crossed with the IP° triple mutant, and ts IP° recombinants were isolated. A ts mutant was picked in each of the early head-formation genes, 20, 21, 22, 23, 24, 31, and 40, the early head-completion group (genes 17 and 49), and some early genes not believed to be involved directly in head formation (genes 41 and 55). Most of the ts IP° mutants produced show little difference in growth at permissive temperature on pm^+ or pm^- bacteria. Differences in burst size between the isogenic pm^+ and pm^- bacteria of the multiple mutants are not apparently significant, since they do not differ appreciably from burst-size differences of the single ts mutant. For a number of the multiple mutants (e.g., 20ts IP°, 23ts IP°), there is a slight increase in

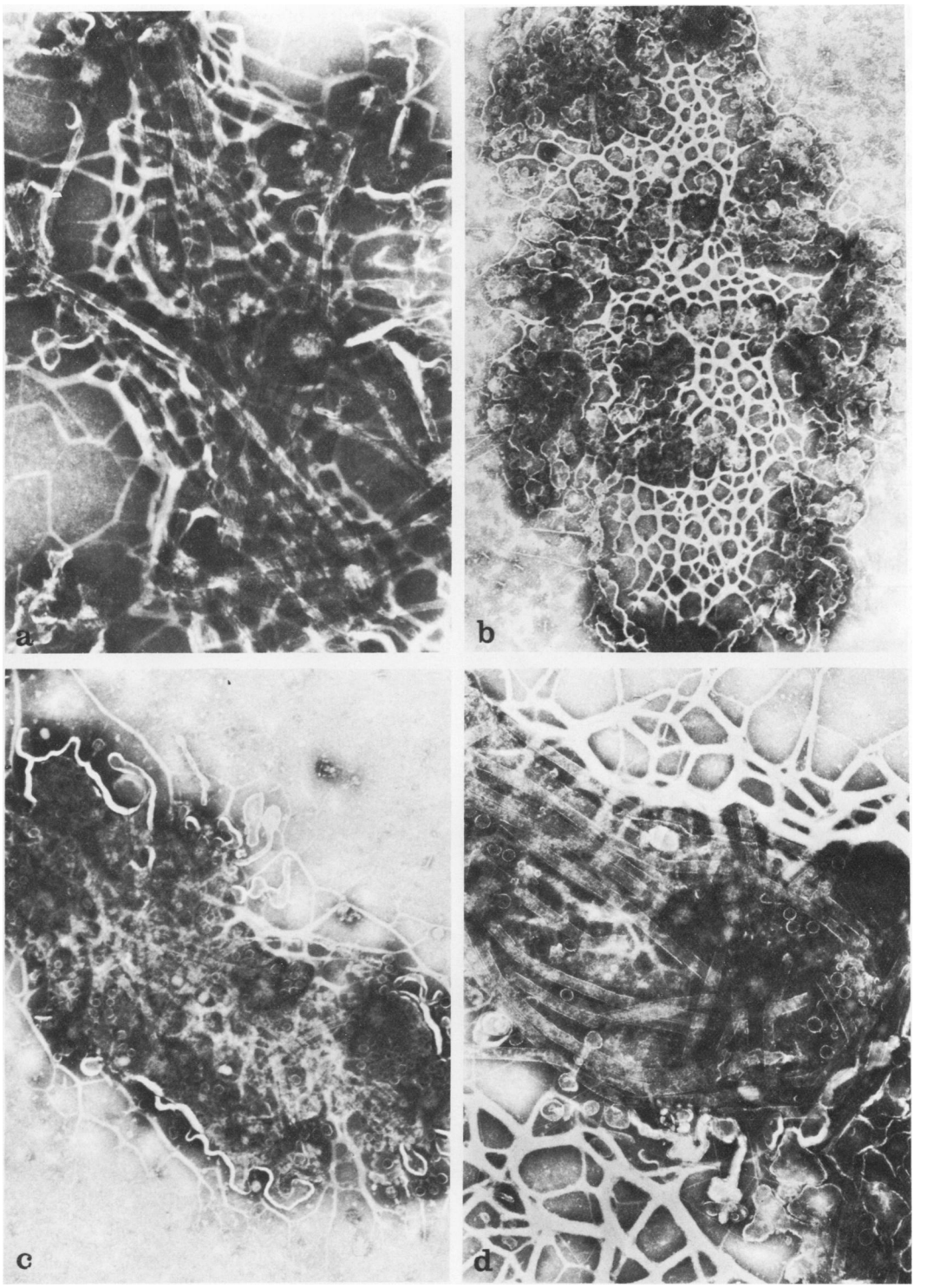

FIG. 3. *Micrographs of in situ lysed cells infected with head gene and head gene-IP° multiple mutants. (3a) 20 (amN50) IP° 40 min after infection, ×25,000; (3b) 21 (amN90) 30 min after infection, ×15,000; (3c) 21 (amN90) IP°, 30 min after infection, ×22,000; (3d) 24 (amN65) IP° 30 min after infection, ×25,000.*

efficiency on growth of pm$^+$ bacteria which is also suggested by the small size of the multiple-mutant plaques on pm$^-$ as compared with pm$^+$ bacteria at 25 C. Nevertheless, the burst-size differences are small, and for all such multiple mutants efficiencies of plating on the pm$^+$ and pm$^-$ bacteria are equal. There is however one exception among the group in Table 3; the mutants 22 tsL147 IP° and 22 tsL147 IPIII$^-$ have plating efficiencies of 10^{-3} on pm$^-$ relative to pm$^+$ bacteria; it is probable that this value underestimates the actual difference, due to the difficulty of growing these two multiple mutants without accumulating large numbers of revertants. Burst-size measurements with ts IP° multiple mutants prepared with both available ts mutants in gene 22 (A74 and L147) also show that growth on pm$^+$ bacteria at 25 C is much better than on pm$^-$ bacteria (Table 3). It appears that the IPIII mutation is primarily involved in the interaction with the ts22 mutants, since, of the single 22ts IP$^-$ mutants, only the 22ts IPIII$^-$ mutant grows better upon suppression of the IP amber mutation.

TABLE 2. *Shift to polyhead production in 21 IP°- and 24 IP°-infected cells*

Cells infected with mutant[a]	No. of cells examined	Polyheads/cell	τ particles/cell	τ particles/polyheads
21$^-$	17	2.7	205.6	76.5
21$^-$ IP°	15	9.4	143.5	15.3
24$^-$	26	15.2	86.5	5.7
24$^-$ IP°	22	21.3	63.0	2.9

[a] Forty minutes postinfection.

(iv) Protein composition and cores of defective heads. Cores were originally visualized by electron microscopy within polyheads and τ particles (14). This internal material often appears relatively unordered upon negative staining, but displays a high degree of organization in thin sections. Evidence was cited that p22 and IPs are found in isolated defective heads produced at high temperature by ts mutants in gene 23 (22). Similarly, internal proteins and p22 can be identified in purified ts23-defective heads or in τ particles as judged by sodium dodecyl sulfate-gel electrophoresis, and by radio-immunodiffusion against anti-

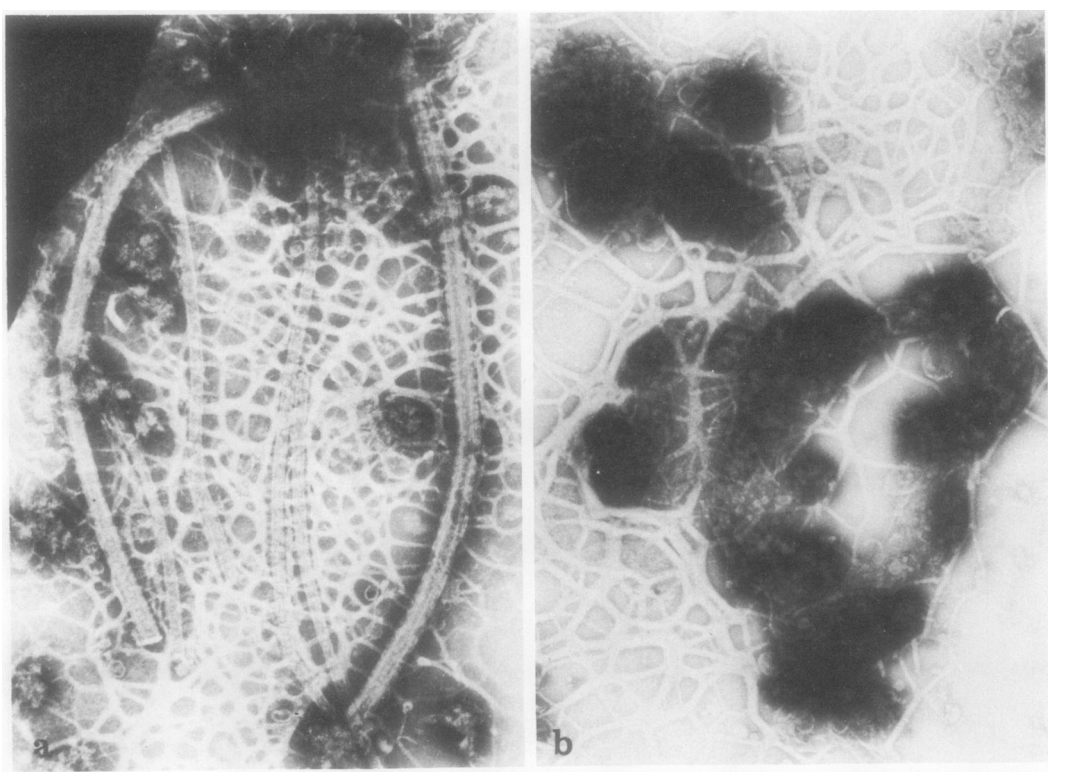

FIG. 4. *Micrograph of in situ lysed cells infected with (4a) 22 (amB270) 40 min after infection; and (4b) 22 (amB270) IP° 40 min after infection.* ×25,000.

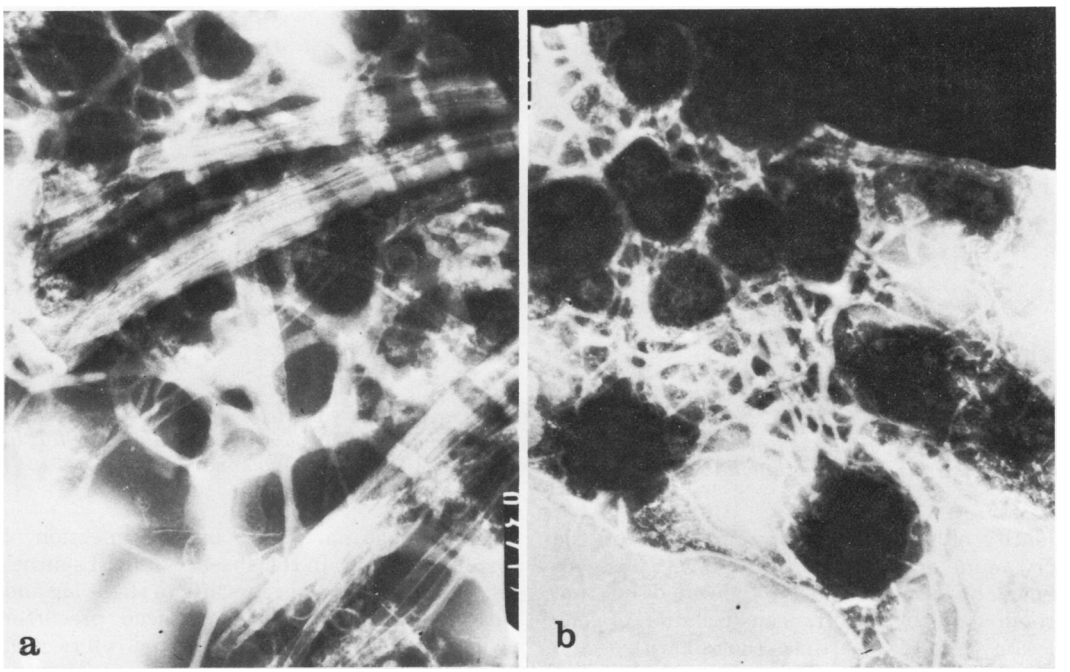

FIG. 5. *Micrograph of in situ lysed cells infected with (5a) 22 (amB270) 90 min after infection, ×40,000; and (5b) 22 (amB270) IP° 90 min after infection, ×30,000.*

TABLE 3. *Interaction between internal protein mutants and ts mutants in other head genes*[a]

Mutant	Defect	Burst size B40su⁺/ burst size P301su	Mutant IP°	Burst size B40su⁺/ burst size P301su
T4⁺		1.0	IP°	1.1
20ts	Head formation	1.1	20ts IP°	1.6
21ts	Head formation	1.1	21ts IP°	1.3
23ts	Head formation	0.9	23ts IP°	1.7
24ts	Head formation	1.0	24ts IP°	1.7
31ts	Head formation	0.8	31ts IP°	0.9
40ts	Head formation	0.9	40ts IP°	1.4
17ts	Head completion	1.3	17ts IP°	1.0
49ts	Head completion	1.2	49ts IP°	1.4
41ts	DNA synthesis	1.1	41ts IP°	1.1
55ts	Maturation deficient	1.2	55ts IP°	1.2
22 tsA74	Head formation	1.2	22 tsA74 IP°	4.0
22 tsL147	Head formation	1.1	22 tsL147 IP°	8.5
			22 tsL147 IPI⁻	0.7
			22 tsL147 IPII⁻	0.4
			22 tsL147 IPIII⁻	10.6

[a] *E. coli* B40su$_I$⁺ or P301 pm⁻ were infected with the ts or ts-IP° multiple mutants listed, at a multiplicity of 0.2, and, after adsorption for 7 min, were treated with T4 antiserum for 5 min and then diluted 10^{-4} in H-broth. Unadsorbed phages, infectious centers, and yield 90 min after the initial infection at 25 or 30 C were measured on B40 to calculate the burst size. The burst sizes of T4 and IP° on su⁻ bacteria were approximately 240 and 150.

bodies directed against the purified internal proteins (Fig. 6a, b). It is apparent from the presence of cleaved p23 that some cleavage has occurred in the isolated 21 (amN90) τ particles, and that there is some contamination by other phage structural components. Nevertheless, p22 and the three internal proteins are high-level components of the isolated τ particles. The

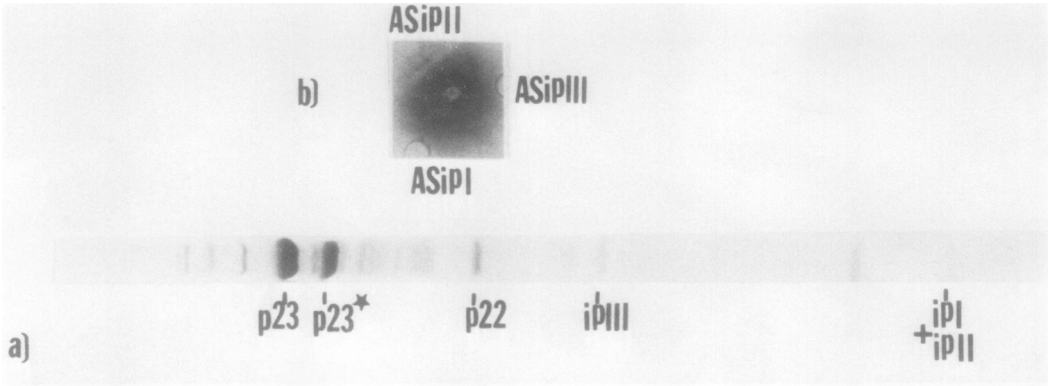

FIG. 6. *Protein composition of purified γ particles. Purified radioactive γ particles were (a) electrophoresed on sodium dodecyl sulfate-polyacrylamide gels or (b) reacted with antisera prepared against purified IPI, IPII, and IPIII. Purification, gel techniques, and radioautography are as described (Materials and Methods, iv).*

identity of the internal protein bands on the acrylamide and immunodiffusion gels was confirmed by electrophoresis of purified internal proteins and by simultaneous isolation of gene 21 (amN90) IP° τ particles (not shown).

In view of the accumulated evidence that cores are likely to be composed largely of p22 and the internal proteins, it was of interest to attempt to visualize cores in defective heads formed in the presence or absence of internal proteins. A large number of sectioning studies was carried out by using the procedure of Séchaud and Kellenberger (21), which is reported to give better preservation of various internal components. Morphologically similar cores exist in the τ particles produced in either 21⁻ or 21⁻ IP° infections (Fig. 7a, b). In the 21⁻ IP° τ particles, the cores frequently give the impression of being less dense than the cores of the 21⁻ τ particles (Fig. 7a, b), but variation in staining could also produce this effect. Cells infected with a ts mutant of gene 23 at high temperature produce aberrant heads which (Fig. 7c) have far less well-defined head and core structure, and 23ts IP° heads have a similar appearance.

DISCUSSION

Our morphological observations are consistent with previous demonstrations that the three major T4 internal proteins are dispensable (4, 22). Mutants defective in synthesis of IPI and IPII do not induce increased defective head formation, as was suggested also by both normal phage yields and head protein cleavage after infection with these mutants. That IPI and IPII do not appear to have major roles in promoting correct head assembly is consistent with the observation that at least IPI functions in the mature phage particle to initiate infection of certain hosts (5). In the absence of IPIII synthesis, phage formation occurs after a slight lag and with reduced efficiency, and head precursor proteins accumulate in the infected cell (4, 22). This can be correlated with the production of many polyheads whose formation starts soon after the normal time of appearance of the first phage particle in a wild-type infection. Deletions of the IPIII gene also appear to induce production of polyheads as well as phages in the infected cell (unpublished observations). An IP° mutant lacking all three major internal proteins did not appear more defective than IPIII⁻ as judged by defective structure accumulation. Cummings et al. (7) reported that an IPIII⁻ mutant was able to form canavanine-induced lollipops (giant phages), whereas IP° was not; this interesting difference is not apparent from defective structure accumulation or other measurements of infected cells under normal growth conditions. It should be noted that an absence of internal proteins from the infected cell is not apparently associated with defective T4 DNA packaging. There is no tendency to accumulate increased numbers of empty phage heads in IP° infections. Rather, defectiveness is manifested at an assembly stage which is generally believed to precede DNA packaging, the formation of the early capsid shell prior to protein cleavage.

Although some τ particles are produced in an IPIII⁻ infection, the mutation seems to be predominantly associated with the production of single-layered polyheads. The number of τ particles produced is not large and is probably consistent with the observation that τ particles are normal intermediates in head formation (23). The same conclusion is drawn from the

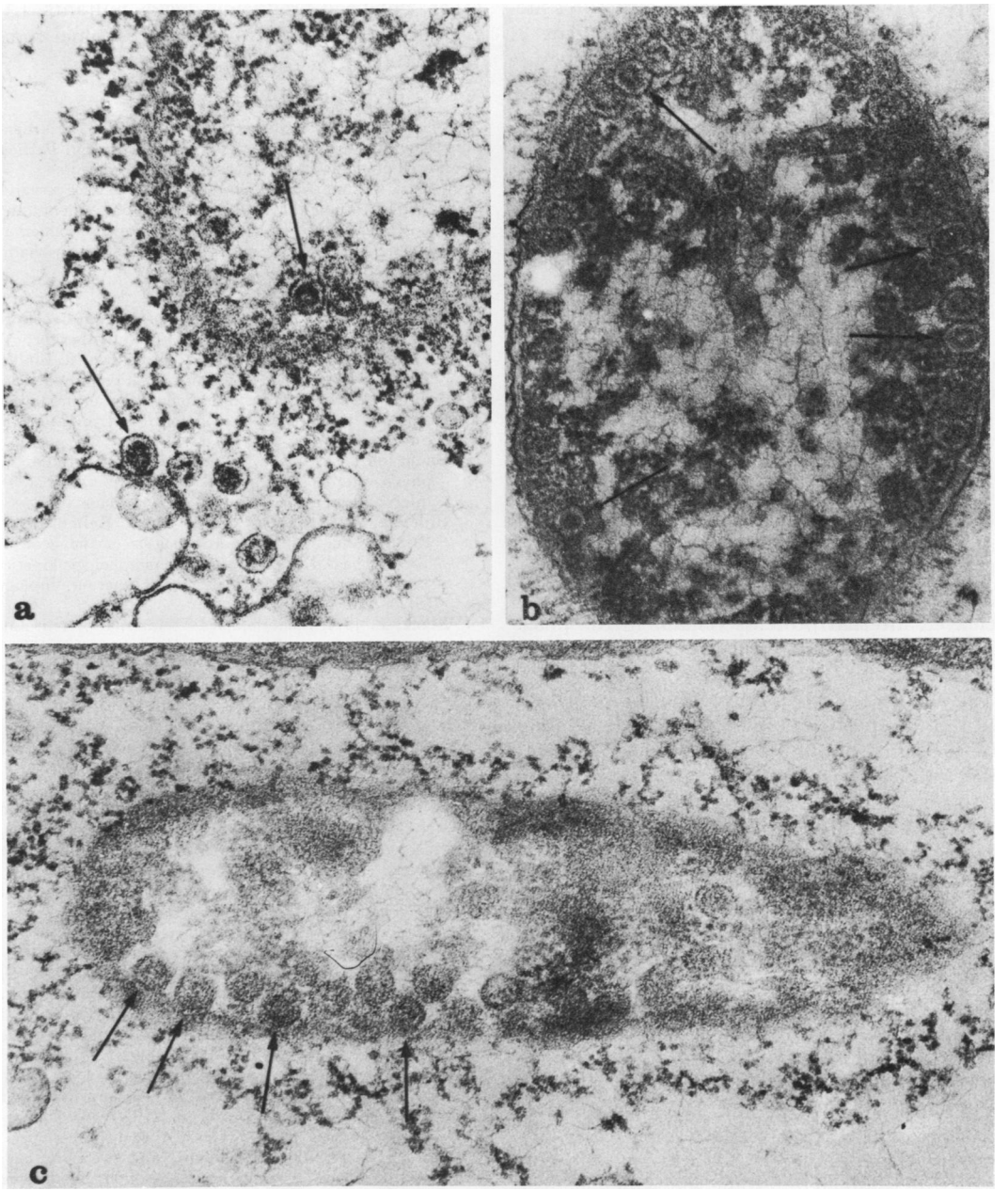

FIG. 7. *Micrographs of thin sections of cells infected with (7a) 21 (amN90); (7b) 21 (amN90) IP°; and (7c) 23 (tsL65). Cells were centrifuged from suspension 45 min after the initial infection and fixed and stained according to Séchaud and Kellenberger (21).* ×*75,000.*

survey of amber early head gene-IP° multiple mutants—the IP° mutations promote polyhead formation where head formation is not blocked at a more defective stage. Thus, in combination with gene 20 and 40 mutants, polyhead formation continues to the exclusion of other aberrant head forms; when combined with gene 21 and 24 mutants, the IP° mutations promote polyhead formation at the expense of τ particle synthesis, whereas with 23 and 31 gene-defective mutants no ordered structures can be formed. The only shift in defective structure formation of qualitative significance in the amY IP° series is with mutants in gene 22 where combination with IP°

causes lump formation-defective structures previously shown to accumulate only in gene 31 mutants. These shifts in defective structure formation are most readily explained by the notion of a T4 assembly core.

Many lines of evidence support the hypothesis that p22 and the internal proteins interact to form a core which is required for assembly of p23 and the other capsid proteins (22). (i) The internal proteins and p22 can be isolated in a protein complex from head-defective-infected cells (22). (ii) The interaction of the ts mutants in gene 22 with the chain termination mutants in the structural genes for the internal proteins (Table 3) suggests the physical interaction of these gene products in vivo. (iii) The internal proteins and p22 have been found in defective ts23 heads (22), τ particles (18) (Fig. 6), and polyheads (20), structures in which morphological cores have been visualized. When assembly continues normally, the core is in part removed, apparently by proteolytic cleavage of p22 to fragments, and removal of a portion of the N-terminal sequences of IPI, IPII, and IPIII; the cleaved internal proteins remain in the phage head in association with DNA (16, 18, 22; A. Tsugita, L. W. Black, and M. K. Showe, J. Mol. Biol., in press). (iv) Lump formation in gene 22 IP°-infected cells can most readily be explained by the absence of any core-related protein with the consequence that p23 aggregates amorphously.

An absence of internal protein synthesis in 21am IP°- or 23ts IP°-infected cells is not associated with gross morphological changes in core structure as seen in thin sections of τ particles or ts23 heads; slight differences in core structure, although a possibility, cannot be convincingly demonstrated (Fig. 7). Because the assembly of the T4 head appears absolutely dependent upon assembly of a core, and the internal proteins are all dispensable, the existence of a morphologically similar core in the IP° infections is not surprising. However, since an absence of IPIII does lead to large-scale polyhead formation, it would appear that core structure is affected in some way which leads to increased aberrant assembly of the capsid precursor. This suggests that correct assembly of the capsid is dependent upon the structure of the core, and that the components of the capsid are not fully shape-specifying. Recent evidence for the same conclusion about the influence of core structure upon head structure is the finding that the polyheads produced by mutants in either gene 22 or IPIII have a different range of diameters from the polyheads produced by the other polyhead-producing head mutants (A. Steven, U. Aebi, and M. Showe, personal communication).

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