Proc. Natl. Acad. Sci. USA
Vol. 74, No. 9, pp. 3652–3656, September 1977
Biochemistry

DNA packaging and the pathway of bacteriophage T4 head assembly

(virus assembly/protein cleavage/DNA synthesis)

C. L. HSIAO AND L. W. BLACK

Department of Biological Chemistry, University of Maryland, School of Medicine, 660 West Redwood Street, Baltimore, Maryland 21201

Communicated by William B. Wood, May 26, 1977

ABSTRACT **A cold-sensitive mutation in the structural gene for a minor phage T4 capsid protein (p20) leads to formation of heads containing p20 and cleaved head proteins and empty of DNA. Such heads can be filled with DNA and converted to active phages *in vivo* upon shift to high temperature. It appears that p20 has two distinct roles in head assembly: first, in construction of the prehead shell (blocked by *ts* and *am* mutation) and, second, in DNA packaging (blocked by *cs* mutation). The latter function is closely associated with gene 17 product, previously known to be required for DNA packaging. Temperature shift studies of *cs-ts* double mutants and other observations allow determination of phage functions required for DNA packaging. Contrary to previous proposals, we find that T4 DNA packaging is not directly coupled to and can follow DNA synthesis, protein cleavage, prehead core removal, and gene 21-mediated cleavage-induced increase in head volume. Our evidence suggests that an altered head assembly pathway exists and that DNA packaging is probably initiated by DNA–capsid (p20) interaction.**

Assembly of the bacteriophage T4 head involves formation of a prehead, cleavage of prehead shell and core proteins, and synthesis, maturation, and packaging of the DNA. The sequence of these processes and their mechanistic relationships are not established. It is now generally accepted that an assembly core-containing prehead is first assembled (1, 2) and then filled with DNA; however, the time of DNA packaging in relationship to core and capsid protein cleavage is disputed. Laemmli identified several prehead intermediates and proposed that the major capsid protein (p23, the protein product of gene 23) is first cleaved (prehead I → II) and then DNA packaging is coupled to core protein cleavage (prehead II → III) (3, 4). On the other hand, it has been suggested by Kellenberger that DNA packaging precedes cleavage of capsid proteins (p23) (5). In this paper we present evidence suggesting that neither of these pathways is correct. The evidence is also against the proposal that filling of T4 heads with DNA depends upon concurrent DNA synthesis (6).

These questions are important because they relate to the function of protein cleavage in virus assembly and the unknown mechanism of DNA condensation. It was initially thought that basic DNA-binding internal proteins of the mature head might condense the DNA, but these proteins proved to be nonessential (1, 7). It was then proposed that DNA packaging was coupled to cleavage of core proteins, so that the generation of acidic internal peptide core remnants might cause repulsive collapse to ψ-like DNA (8, 9). Similarly, packaging could be coupled to exit of core proteins from the prehead in phages lacking protein processing (10). Another general idea is that packaging is coupled to the expansion of the capsid shell which accompanies protein processing and head filling (11, 12).

In this paper we characterize a cold-sensitive (*cs*) mutation in the structural gene for a minor capsid protein (p20) with a novel morphological phenotype: empty, fully cleaved capsids, instead of polyheads, are produced. Order of function studies with recombinants with temperature-sensitive (*ts*) mutations in other head genes show that DNA packaging into the *cs* 20 heads proceeds when either protein cleavage or DNA synthesis is blocked, and suggest a partial assembly pathway for T4 head formation.

MATERIALS AND METHODS

Bacteria and bacteriophage T4 mutants used are listed in Fig. 5 or were as described (13). *Escherichia coli* DF2000 (phosphoglucose isomerase$^-$, glucose-6-*P* dehydrogenase$^-$) is unable to synthesize or metabolize glucose (14). *E. coli* CR63 Su_I^+ r_6^- $r_{2,4}^-$ (Helen Revel) is permissive for amber (*am*) mutants and nonglucosylated phages. Sodium dodecyl sulfate/polyacrylamide gradient (9–13%) gel electrophoresis with a discontinuous buffer system (15) was used.

RESULTS

Mutations in the group of genes (Y-genes) involved in forming the capsid are usually gene specific with respect to the type of defective head structure that is produced (16). Thus all *am* and *ts* mutations located in gene 20 give rise to open, single-layered, tubular polyheads. These mutations also block cleavage of head precursor proteins *in vivo*, as do other Y-gene group mutations (15). Therefore, it was surprising that a newly isolated *cs* mutation located in gene 20 did not significantly prevent protein cleavage *in vivo* under nonpermissive conditions (unpublished, see Fig. 4*k*) and was not associated with polyhead production, but gave rise to empty head structures (Fig. 1). The phenotype of this *cs*20 mutation is similar to that of gene 17 mutations. Indeed, the *cs*20 mutation and gene 17 function appear very closely interrelated because of (*i*) similar morphological phenotypes (empty head and ghost production); (*ii*) accumulation of similar sized concatameric T4 DNA (unpublished); (*iii*) incompatibility in double mutant combination of the *cs*20 mutation and any *ts* or *am* mutation tested in gene 17; and (*iv*) second site mutations situated in gene 17 (R1) which suppress the *cs*20 phenotype. The most reasonable interpretation is that p20 and p17 interact directly and function at the same step in head formation, probably the DNA packaging stage, as was thought to be the case for gene 17 (18).

Thin sections of bacteria infected with *cs*20 at nonpermissive temperature reveal many empty heads in the interior of the cell (Fig. 1). These clearly differ from the protein core-containing membrane-associated Tau-particles which are also seen in small numbers and are known to be intermediates in head formation (2, 19). The *cs*20 heads appear to be empty of DNA or core material, and to be of roughly the same size as the occasional head fully packaged with DNA seen in growth under slightly leaky conditions (24°) (Fig. 1). There do not, however, appear to be heads with intermediate amounts of DNA in such sections.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: p23, the protein product of gene 23; $p23^C$, cleaved product of gene 23 found in the mature head; *ts*, temperature sensitive; *cs*, cold sensitive; *am*, amber.

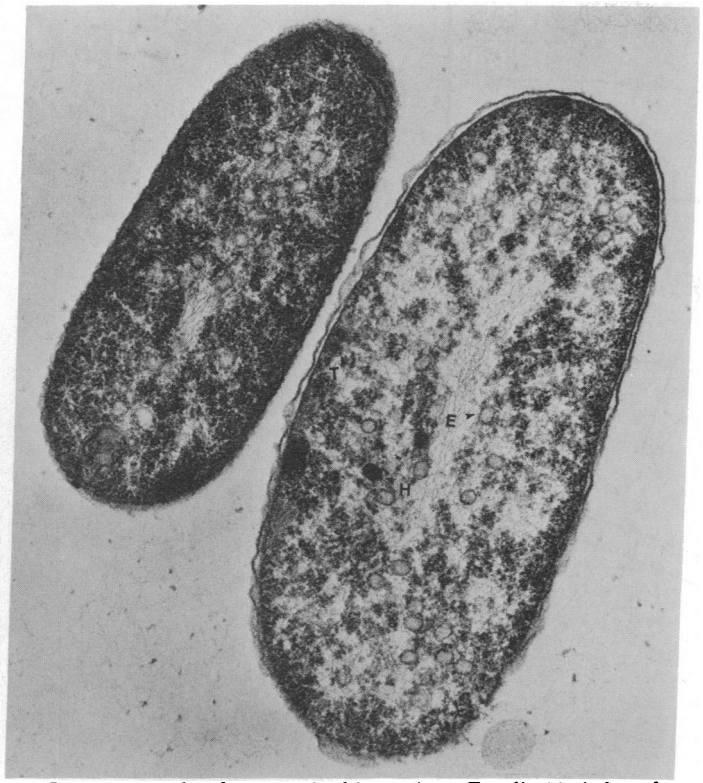

FIG. 1. *cs*20 heads as seen in thin sections. *E. coli* p301 infected with *cs*20 at 24° for 96 min were prepared for thin sections according to Kellenberger *et al.* (17). T, Tau particle; E, empty head; H, DNA-filled head.

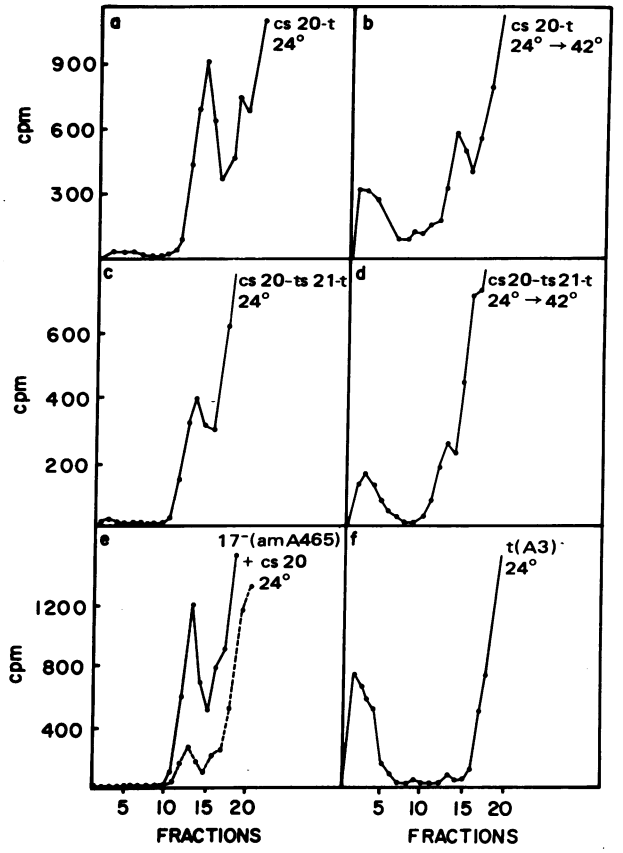

FIG. 2. Maturation of *cs*20 empty heads. *E. coli* p301 were infected with a multiplicity of 5 at 24°, labeled with ^{14}C-labeled amino acids (1 μCi/ml) at 30 min, and chased at 37 min with 0.2 ml of 20% casamino acids. Half of the culture was then shifted up to 42° at 60 min for 30 min. The lysates were then analyzed on 10–30% sucrose gradients in phosphate buffer. Centrifugation was in an SW50.1 rotor, at 22,000 rpm, at 4° for 50 min. Fractions were collected from the bottom of the tube and 25 μl per fraction was taken for determination of radioactivity. Gradient *e* contains ^{3}H-labeled *cs*20 particles (---) as a marker, which bands at the same position as the gene 17 empty heads.

When the heads produced in the *cs*20 mutant infection are analyzed on sucrose gradients, it is apparent that these heads have the sedimentation properties characteristic of gene 17 defective empty heads (Fig. 2 *a* and *e*). In contrast to reported properties of gene *ts* 17 defective heads (6), however, the *cs*20 heads can be converted to full heads upon shift up to high temperature (Fig. 2 *a* and *b*). That the full heads produced do not result from *de novo* assembly of head precursors is demonstrated by the disappearance of the *cs*20 empty heads upon shift to high temperature (approximately 40% conversion) (Fig. 2 *a* and *b*), production of phages when the shift is carried out in chloramphenicol (Fig. 3), and nearly equivalent conversion with the multiple mutant *cs*20-*ts*21-*am*t† (Fig. 2 *c* and *d*). In this multiple mutant, protein cleavage and maturation beyond the prehead stage are blocked by the *ts* mutation after the shift to 42°. The *cs*20 defect does not prevent protein cleavage *in vivo*, and the empty *cs*20 heads contain fully cleaved head proteins and an apparently normal amount of p20, resembling gene 17 defective heads (see Fig. 4 *j* and *k*).

When *cs*20-infected bacteria are shifted to 42°, there is a very rapid formation of viable phage (Fig. 3). The yield is highly dependent upon the growth conditions and the time of shift-up to high temperature. When temperature shift-up of *cs*20 is carried out in bacteria grown at 24°, an increase of 60 phages is obtained, whereas at 20°, the burst is significantly lower (Fig. 3); however, this reflects poor T4 growth at 20°, and the yield generally approximates 20–40% of the wild-type yield. Shift-up to higher temperatures at later times (Fig. 3, curves *b* and *c*, and unpublished) results in increasingly poor yields. This could be due to formation of ghosts from maturable *cs*20 heads at later times, in keeping with the fairly large proportion of ghosts seen in lysates *in situ*, but this has not been carefully examined.

† *am*t prevents lysis of infected nonpermissive cells before addition of chloroform (20).

Although the data already presented strongly suggest that the *cs*20 heads are empty of DNA *in vivo*, it could be supposed that DNA packaging has already proceeded to a significant extent, but that such intermediately packaged structures are neither stable to isolation nor revealed by electron microscopy. An experiment that argues against this possibility is presented in Fig. 3. When *cs*20-*ts*21-*am*t is grown in *E. coli* DF2000 in the absence of added glucose, newly synthesized T4 DNA cannot be glucosylated (*Materials and Methods*). However, when glucose is added to the infected cells, it is rapidly taken up and utilized to glucosylate the DNA pool. When glucose is added at the moment of the temperature shift up to 42°, the phages produced are fully glucosylated, as judged by their ability to grow on hosts that restrict nonglucosylated phages. When glucose is added later after the shift, however, the proportion of glucosylated phages in the final burst decreases exponentially (Fig. 3, curves *f* and *g*). Therefore, the DNA that is incorporated into the *cs*20 heads is exposed to the T4 glucosylation system at the moment of temperature shift-up. It appears unlikely that phages containing a significant complement of condensed but unstably incorporated DNA would be accessible to glucosylation, and it is likely that a significant stretch of nonglucosylated DNA will subject a phage to restriction (21). Support for this idea is provided by an analogous experiment with *ts*49(C9). When DF2000 infected with *ts*49(C9) is shifted down to low temperature in chloramphenicol with glucose addition at the moment of shift-down, we found

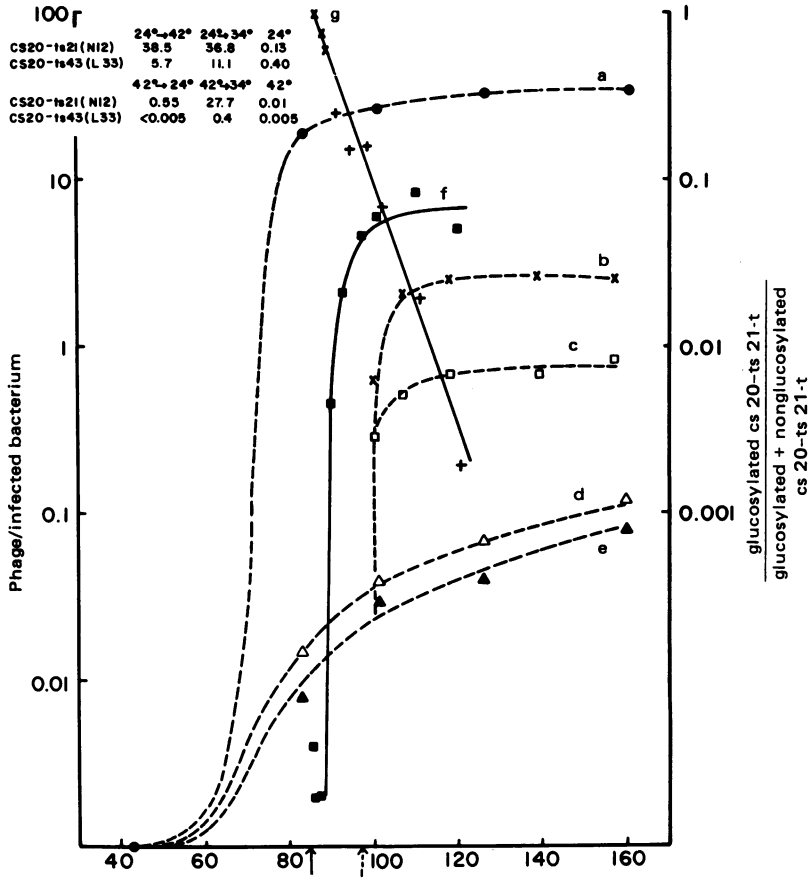

FIG. 3. DNA packaging into *cs*20 heads. *E. coli* P301 growing in H-broth at 20° were infected with phage at a multiplicity of 5. The phage yield is measured after the indicated times at 20° or at 20° then at 42° (times of temperature shift are indicated by arrows) as phage/infected bacterium. (*a*) T4D$^+$, 20°; (*b*) *cs*20-t shifted to 42° at 97 min; (*c*) *cs*20-t shifted to 42° at 97 min, 200 μg of chloramphenicol added per ml at 95 min; (*d*) *cs*20-t grown at 20°; (*e*) *cs*20-*ts*21-t grown at 20°. DF2000 in tryptone broth +M9 salts infected with *cs*20-*ts*21-t, were grown at 20° for 85 min and then shifted to 42°. The phage yield (*f*) was measured as phage/infected bacterium at the indicated times on CR63r$^-$r$^-$. At the indicated times glucose was added to 0.4% portions of the same infected culture; after 40 min at 42° the ratio of glucosylated to nonglucosylated phages in the final yield was determined by growth on B40 (restricts nonglucosylated phages) and CR63r$^-$r$^-$. (*g*) *cs*20-*ts*21-t/(*cs*20-*ts*21-t* + *cs*20-*ts*21-t). (*Inset*) Order of function by shift-up and shift-down experiments with double *cs-ts* mutants. The yield per cell was measured after shift to 42° for 30 min, 34° for 45 min, or 24° for 60 min. Shift-up was at 60 min and shift-down at 15 min after infection.

that only 10% of the phages produced escape restriction. *ts*49 heads are believed to be only partially filled due to failure to remove recombinant joints from the concatameric DNA pool (22). The different *ts*49 and *cs*20 responses to glucosylation support the idea that partially incorporated DNA is inaccessible to glucosylation.

By construction of double mutants combining *cs* and *ts* mutations in different genes, the order in a developmental pathway of functioning of the two gene products can be inferred by temperature shift-up and shift-down experiments (23). Since we have already demonstrated that the *cs*20 developmental block is in the T4 DNA packaging step, combination of *cs*20 with *ts* mutations in other T4 genes could reveal the dependence of packaging upon other phage functions. Detailed results for two such double mutant combinations are presented (Fig. 3, *inset*). From these results it can be inferred that the *cs*20 block follows that of both *ts*43 (L33-DNA polymerase) and *ts*21 (N12-head assembly protease). In addition, when DNA synthesis in *ts*43 (L33) infection is examined, it can be determined that shift of infected cells to 42° results in a rate of DNA synthesis 1% of wild type within 2 min; data, not presented, are similar to those of others (24). Therefore, it appears that the *ts*43 (L33) DNA polymerase already synthesized at low temperature is inactivated when shifted to high temperature more rapidly than the kinetics of *cs*20 head filling (Fig. 3). It appears that DNA synthesis can be very greatly inhibited without greatly diminishing the yield of phages. Similarly we have examined *ts* mutations in gene 21, the structural gene for the T4 head assembly protease (25). We find that shift of *ts*21 from 24° to 42° results in a sudden cessation of protein cleavage of precursors synthesized either before (Fig. 4 *d–g*) or after the temperature shift (Fig. 4*a*), suggesting that the *ts*21 protease is rapidly inactivated at high temperature. Note that head filling apparently continues for some time after shift of *ts*21 to 42°, at a time when cleavage of head precursors (e.g., IpIII → IpIII^c) synthesized and incorporated into structures before shift-up is no longer increasing (Figs. 3 and 4 *d–g*).

Since the *ts*21 mutation prevents maturation of head precursors synthesized at high temperature, we can ask which proteins synthesized after temperature shift-up are incorporated into the *cs*20-*ts*21-*amt* heads assembled prior to the shift up. Certain newly synthesized proteins are added as the *cs*20-*ts*21-*amt* particles are converted to phages at 42°. Chief among these are the soc protein of the head (26), p24 (Fig. 4; unpublished), and tail and fiber proteins (Fig. 4 *h* and *i*). It is important to note that no p20 or internal proteins appear to be incorporated into the *cs*20 heads after shift-up.

Fig. 5 summarizes temperature shift experiments of the type reported in Fig. 3, utilizing *cs*20 and *ts* mutations in various genes. Formal analysis of these results (Fig. 5*A*) conforms to other knowledge about the functions of these genes in T4 development (Fig. 5*B*); e.g., gene 13 is known to be involved in

formation of the collar that attaches head and tail, and fibers are believed to be synthesized by an independent assembly pathway (10, 27).

DISCUSSION

Our order of function analyses suggest that the product of gene 20, a minor, essential capsid protein, has two separated functions in the head assembly pathway. First, p20 acts in formation of the prehead shell; second, p20 acts in packaging the DNA into the processed shell (Fig. 5). *am* and *ts* mutations in gene 20, which eliminate the first function, block protein cleavage and lead to assembly of polyheads, an aberrant prehead structure. Since the polyhead formation of such gene 20 mutations is dominant in double mutant combinations with mutations in many of the other Y-genes (16), p20 is likely to act quite early in the assembly of the prehead. In contrast, the defectiveness of the *cs*20 we have discovered is late: protein cleavage proceeds, no polyheads are formed, and empty cleaved heads containing p20 accumulate in the infected cell. These heads can be filled with DNA and converted to active phages once the late p20 function is restored by shift to high temperature. Gene 17 function appears to be implicated in this DNA packaging step. Although p17 has not been identified, it has recently been reported that gene 17 may control the activity of a head-associated endonuclease (28).

There is a long-standing.controversy over the existence of labile DNA-filled intermediates in head formation (5, 17). Our evidence strongly suggests that the *cs*20 heads are actually empty of DNA *in vivo*: (*i*) Thin sections of *cs*20 infected cells reveal apparently completely empty shells (Fig. 1). (*ii*) Isolated *cs*20 heads are empty, as analyzed on sucrose gradients, and mature into phage upon shift to high temperature (Fig. 2), (*iii*) *cs*20 DNA is concatameric. (*iv*) The order of function studies (Fig. 5) suggest that T4 DNA ligase must act after release of the *cs*20 block, since neither temperature shift is productive. This could be explained from knowledge that late T4 DNA is nicked (29), and should be repaired prior to entry into the head to yield viable phage. Perhaps most convincing, (*v*) the DNA that is to be packaged into the *cs*20 heads remains accessible to the T4 glucosylation system in the infected cell at the moment of the temperature shift (Fig. 3, curves *f* and *g*). This is in contrast to partially filled *ts*49 heads, in which the partially packaged DNA is mainly unable to escape restriction when glucosylation is allowed to proceed at the moment of release of the *ts*49 block (see *Results*).

The order of function studies and biochemical characterization of gene 43 and 21 *ts* mutants suggest that DNA packaging occurs after and in the absence of concurrent DNA synthesis or protein cleavage. Therefore, our results also suggest a head assembly pathway (Fig. 5*B*) in which, after construction of the assembly-core containing prehead, shell (p23) and core components (p22 and internal proteins) are cleaved. After cleavage, DNA is introduced into the capsid and then packaged.

We now discuss more speculatively our findings in relation to the structure of the T4 head and the mechanism of DNA packaging. It appears unlikely from our results that either DNA synthesis or protein cleavage provides the driving force for DNA condensation (3, 6, 8). However, it also has been suggested that p21-induced cleavage of capsid proteins causes major changes in the surface lattice structure and an increase in head volume (30). If this is the case, the p21 cleavage-induced volume changes are not directly coupled to DNA packaging, as is also suggested by comparison of the sizes of the full and empty *cs*20 heads (Fig. 1). However, this important point remains to be studied by more careful measurements of head size. Our order of function studies also argue that removal and cleavage of the

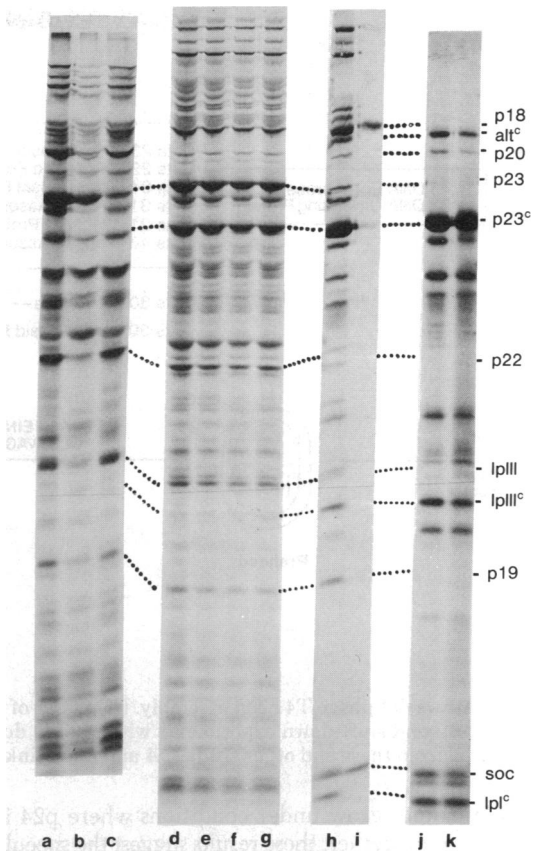

FIG. 4. Analysis on polyacrylamide gradient gels of ^{14}C-labeled lysates (*a–g*), CsCl block gradient-purified phages (*h* and *i*), and empty heads (*j* and *k*). (*a* and *b*) Head precursor proteins synthesized at 42° in *cs*20-*ts*21-*am*t infected cells were not cleaved: (*a*) P301 was infected at 23° and shifted to 42° at 60 min. It was then pulse-labeled at 61 min, chased at 72 min, and harvested at 78 min. No cleavage of head precursor proteins (e.g., p23 and IpIII) was observed. (*b*) P301 was infected at 42°, pulse-labeled at 8 min, chased at 10 min, and shifted down to 23° at 18 min for 40 min. No cleavage of precursor proteins was observed. (c) *am*23 (H11). (*d–g*) Cleavage of precursor proteins in a *ts*21 infection is stopped immediately upon temperature shift-up: a *cs*20-*ts*21-*am*t infected culture at 20° was labeled between 70 and 80 min and shifted to 43° with chase at 80 min. Sampling at 79 min (*d*), 81 min (*e*), 85 min (*f*), and 90 min (*g*). (*h*) Purified phages. (*i*) Proteins incorporated into *cs*20 heads after temperature shift-up: *cs*20-*ts*21-*am*t infected culture at 24° was shifted to 42° at 60 min and labeled from 66 min, and phages were purified from the shifted culture. (*j* and *k*) Protein composition of empty heads purified from *am*17 *am*10 *am*57 (*j*) and *cs*20 *am*10 *am*57 (*k*) infected cultures labeled continuously at 23.5°.

core are not directly linked to the packaging stage of head maturation (10), as does the observation that the *cs*20 heads already contain fully cleaved p22 and internal proteins (Fig. 4*k*).

Perhaps the most significant observation is that *cs* p20, which is already a part of the capsid shell, can apparently undergo some structural transformation that initiates DNA packaging, suggesting that p20 plays an important role in this process.

What is known of the structural role of p20 in the capsid? (*a*) Measurements suggest that p20 is an integral part of the capsid and that there are about 12 molecules (31). This number does not increase in giant-headed phages, suggesting that p20 is located in the hemispherical caps (32). (*b*) p20 may be important in initiating head formation with assembly of a cap (16). (*c*) *am*17 and *cs*20 prevent pwac attachment to the head (unpublished), suggesting that at least some p20 and p17 are localized at the head tail junction. (*d*) p20 and p24 appear to interact in the head structure, because the multiple mutant *cs*20-

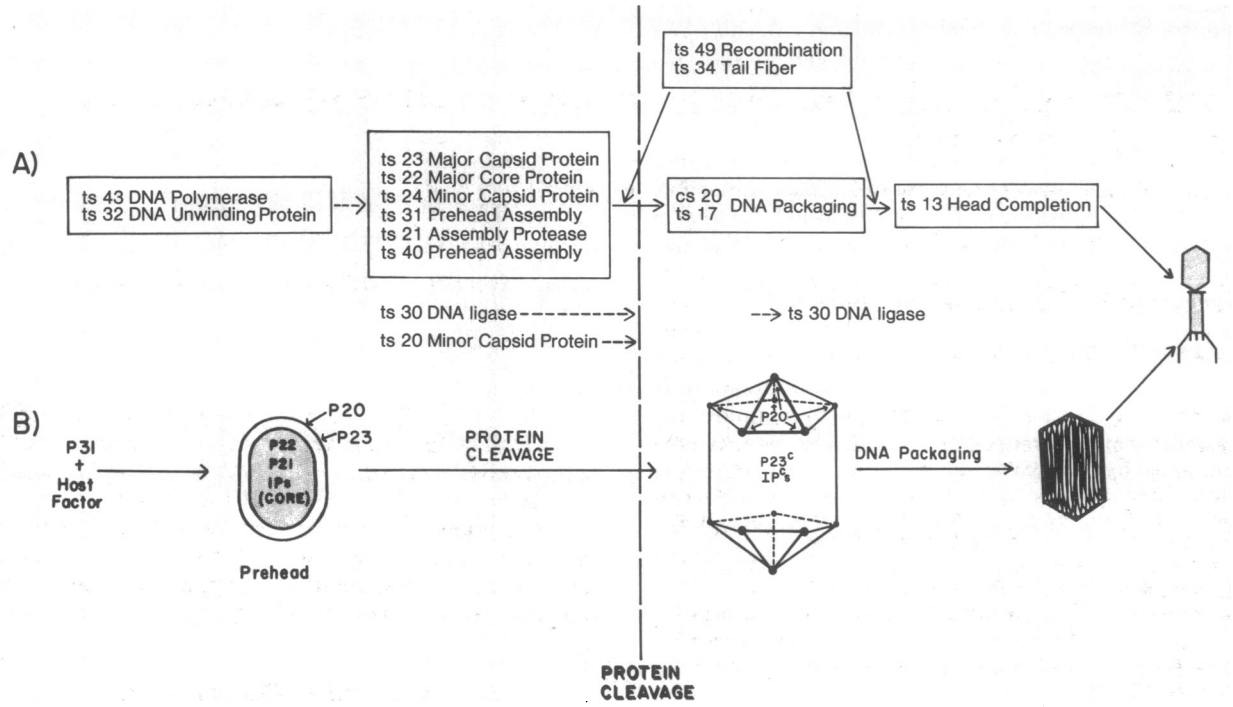

FIG. 5. Pathway of phage T4 head assembly. (*A*) Order of function of gene products in phage T4 head development as inferred from temperature shift-up and shift-down experiments with *cs*20-*ts* double mutants (e.g., Fig. 3, *inset*). (*B*) Structural development of the phage T4 head as inferred from these and other studies. *A* and *B* are linked by the knowledge that *cs*20 is blocked after protein cleavage but before DNA packaging.

am24byp24 cannot grow under conditions where p24 is not produced. Taken together, these results suggest the speculation that p20 is localized at the vertex located at the head-tail junction, and probably at each of the 12 vertices of the isosahedral head.

If this is the case, our work suggests that a protein located at the vertices of the head could have an important role in DNA packaging, perhaps by binding DNA strongly in a late stage of head assembly. Two observations are consistent with this hypothesis. First, we have observed in preliminary experiments with DNA cellulose chromatography that p20 may be a strong DNA-binding protein (unpublished). Second, interesting electron micrographs of partially filled *ts*49 heads suggest areas of condensed DNA localized around the vertices (22). It is possible that after protein cleavage, p20 situated at the vertices is exposed and binds DNA. Binding of DNA to p20, and to some extent to other capsid proteins, could distribute the DNA over the inner surface of the capsid and condense the DNA within the head.

Whether or not these latter speculations are correct, our observations suggest that DNA condensation is probably at least initiated by DNA–capsid (p20) interactions, and that DNA synthesis and protein cleavage are not directly coupled to the DNA packaging process in phage T4.

We thank Dr. David Silverman and Christine Meyer for generous assistance with thin sections. We acknowledge support of National Institutes of Health Grant AI-11676, and the University of Maryland for funds for travel to Snowbird, Utah, September, 1976, where this work was presented at The 5th Biennial Conference on Bacteriophage Assembly.

1. Showe, M. K. & Black, L. W. (1973) *Nature New Biol.* **242**, 70–75.
2. Simon, L. D. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 907–911.
3. Laemmli, U. K. & Favre, M. (1973) *J. Mol. Biol.* **80**, 575–599.
4. Laemmli, U. K., Teaff, N. & D'Ambrosia, J. (1974) *J. Mol. Biol.* **88**, 749–765.
5. Kellenberger, E. (1972) in *Polymerization in Biological Systems*, ed. Wolstanholme, G. E. W. (Scientific Publishers, Amsterdam), pp. 189–206.
6. Luftig, R. & Lundh, N. (1973) *Virology* **51**, 432–442.
7. Black, L. W. (1974) *Virology* **60**, 166–197.
8. Laemmli, U. K., Paulson, J. & Hitchins, V. (1974) *J. Supramol. Struct.* **2**, 276–301.
9. Lerman, L. S. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 1886–1890.
10. Casjens, S. & King, J. (1975) "Virus Assembly," *Annu. Rev. Biochem.* **44**, 555–611.
11. Hohn, B., Wurtz, M., Klein, B., Lustig, A. & Hohn, T. (1974) *J. Supramol. Struct.* **2**, 302–317.
12. Serwer, P. (1975) *J. Mol. Biol.* **92**, 433–448.
13. Castillo, C., Hsiao, C. L., Coon, P. & Black, L. (1977) *J. Mol. Biol.* **110**, 585–601.
14. Fraenkel, D. G. (1968) *J. Biol. Chem.* **243**, 6451–6459.
15. Laemmli, U. K. (1970) *Nature* **227**, 680–685.
16. Laemmli, U. K., Molbert, F., Showe, M. K. & Kellenberger, F. (1970) *J. Mol. Biol.* **49**, 99–113.
17. Kellenberger, E., Eiserling, F. A. & Boy de la Tour, F. (1968) *J. Ultrastruct. Res.* **21**, 335–360.
18. King, J. (1968) *J. Mol. Biol.* **32**, 231–262.
19. Bijlenga, R. K. L., Scraba, D. & Kellenberger, E. (1973) *Virology* **56**, 250–267.
20. Josslin, R. (1970) *Virology* **40**, 719–726.
21. Revel, H. R. & Luria, S. F., (1970) *Annu. Rev. Genet.* **4**, 177–192.
22. Kemper, B. & Brown, D. T. (1976) *J. Virol.* **18**, 1000–1015.
23. Jarvik, J. & Botstein, D. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2046–2050.
24. Curtis, M. & Alberts, B. (1976) *J. Mol. Biol.* **102**, 793–816.
25. Showe, M., Isobe, F. & Onorato, L. (1976) *J. Mol. Biol.* **107**, 35–54.
26. Ishii, T. & Yanagida, M. (1975) *J. Mol. Biol.* **97**, 655–660.
27. Coombs, D. H. & Eiserling, F. A. (1977) *J. Mol. Biol.*, in press.
28. Minagawa, T. (1977) *Virology* **76**, 234–245.
29. Cascino, A., Riva, S. & Geiduschek, E. P. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 213–220.
30. Steven, A. C., Couture, F., Aebi, U. & Showe, M. K. (1976) *J. Mol. Biol.* **106**, 187–221.
31. Aebi, U., Bijlenga, R., Broek, J. vd, Eiserling, F., Kellenberger, E., Mesyanzhinov, V., Müller, L., Showe, M., Smith, R. & Steven, A. (1974) *J. Supramol. Struct.* **2**, 253–275.
32. Bijlenga, R., Aebi, U. & Kellenberger, E. (1976) *J. Mol. Biol.* **103**, 469–498.