DNA packaging and the pathway of bacteriophage T4 head assembly

(virus assembly/protein cleavage/DNA synthesis)

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ABSTRACT A cold-sensitive mutation in the structural gene for a minor phase 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 mutations) 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 intestinal 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 (phosphoglucone isomerase, glucose-6-P dehydrogenase) is unable to synthesize or metabolize glucose (14). E. coli CR63 Su+ r0− r3,4− (Helen Revel) is permissive for amber (am) mutants and nonglucosylated phages. Sodium dodecyl sulfate/polyacryl- amide gradient (9–18%) gel electrophoresis with a discontinuous buffer system (15) was used.

RESULTS

Mutations in the group of genes (Y-genpes) 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. 4k) and was not associated with polyhead production, but gave rise to empty head structures (Fig. 1). The phenotype of this cs20 mutation is similar to that of gene 17 mutations. Indeed, the cs20 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 cs20 mutation and any ts or am mutation tested in gene 17; and (iv) second site mutations situated in gene 17 (R1) which suppress the cs20 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 cs20 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 cs20 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.

Abbreviations: p23, the protein product of gene 23; p23c, cleaved product of gene 23 found in the mature head; ts, temperature sensitive; cs, cold sensitive; am, amber.
When the heads produced in the cs20 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. 2a and e). In contrast to reported properties of gene ts 17 defective heads (6), however, the cs20 heads can be converted to full heads upon shift up to high temperature (Fig. 2a and b). That the full heads produced do not result from de novo assembly of head precursors is demonstrated by the disappearance of the cs20 empty heads upon shift to high temperature (approximately 40% conversion) (Fig. 2a and b), production of phages when the shift is carried out in chloramphenicol (Fig. 3), and nearly equivalent conversion with the multiple mutant cs20-ts21-amt (Fig. 2c 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°C. The cs20 defect does not prevent protein cleavage in vivo, and the empty cs20 heads contain fully cleaved head proteins and an apparently normal amount of p20, resembling gene 17 defective heads (see Fig. 4j and k).

When cs20-infected bacteria are shifted to 42°C, 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 cs20 is carried out in bacteria grown at 24°C, an increase of 60 phages is obtained, whereas at 20°C, the burst is significantly lower (Fig. 3); however, this reflects poor T4 growth at 20°C, 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 cs20 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.

1. amt prevents lysis of infected nonpermissive cells before addition of chloroform (20).
that only 10% of the phages produced escape restriction. ts49 heads are believed to be only partially filled due to failure to remove recombinant joints from the concatameric DNA pool (22). The different ts49 and cs20 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 cs20 developmental block is in the T4 DNA packaging step, combination of cs20 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 cs20 block follows that of both ts43 (L33 DNA polymerase) and ts21 (N12-head assembly protease). In addition, when DNA synthesis in ts43 (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 ts43 (L33) DNA polymerase already synthesized at low temperature is inactivated when shifted to high temperature more rapidly than the kinetics of cs20 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 ts21 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. 4a), suggesting that the ts21 protease is rapidly inactivated at high temperature. Note that head filling apparently continues for some time after shift of ts21 to 42°, at a time when cleavage of head precursors (e.g., IpIII → IpIV') synthesized and incorporated into structures before shift-up is no longer increasing (Figs. 3 and 4 d–g).

Since the ts21 mutation prevents maturation of head precursors synthesized at high temperature, we can ask which proteins synthesized after temperature shift-up are incorporated into the cs20-ts21-amt heads assembled prior to the shift up. Certain newly synthesized proteins are added as the cs20-ts21-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 cs20 heads after shift-up.

Fig. 5 summarizes temperature shift experiments of the type reported in Fig. 3, utilizing cs20 and ts mutations in various genes. Formal analysis of these results (Fig. 5A) conforms to other knowledge about the functions of these genes in T4 development (Fig. 5B); 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 cs20 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-associating endonuclease (28).

There is a long-standing controversy over the existence of lable DNA-filled intermediates in head formation (5, 17). Our evidence strongly suggests that the cs20 heads are actually empty of DNA in vivo: (i) Thin sections of cs20 infected cells reveal apparently completely empty shells (Fig. 1). (ii) Isolated cs20 heads are empty, as analyzed on sucrose gradients, and mature into phage upon shift to high temperature (Fig. 2), (iii) cs20 DNA is concatameric. (iv) The order of function studies (Fig. 5) suggest that T4 DNA ligase must act after release of the cs20 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 cs20 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 cs20 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 ts49 block (see Results).

The order of function studies and biochemical characterization of gene 43 and 20 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. 5B) 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 cs20 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 core are not directly linked to the packaging stage of head maturation (10), as does the observation that the cs20 heads already contain fully cleaved p22 and internal proteins (Fig. 4k).

Perhaps the most significant observation is that 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) am17 and cs20 prevent pwa 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 cs20-
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 ts49 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.

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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 cs20-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 cs20 is blocked after protein cleavage but before DNA packaging.
