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Assembly of Bacteriophage φX174: Identification of a Virion Capsid Precursor and Proposal of a Model for the Functions of Bacteriophage Gene Products During Morphogenesis

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A capsomeric structure sedimenting with an S value of 108 in sucrose gradients was isolated from *Escherichia coli* infected with bacteriophage φX174. The 108S material contained viral proteins F, G, H, and D, and the relative amounts of these proteins in the 108S material were similar to those in the infectious 132S particle, which has previously been described as a possible intermediate in the assembly of 114S phage particles. Electron micrographs indicated that the size and shape of the 108S material resemble those of the 132S particle. The 108S material contained no DNA, and its formation occurred independently of DNA synthesis. The 108S material accumulated in infected cells when viral DNA replication was prevented either by mutation in phage genes A or C or by removal of thymidine from a culture infected with wild-type phage or with a lysis gene E mutant. Upon restoration of thymidine to cells infected with the lysis gene E mutant and then starved of thymidine, the accumulated 108S material was converted to 132S particles and to 114S phage particles, implying that the 108S material is a precursor of phage particles. A model that proposes possible functions for the products of φX174 genes A, B, C, D, F, and G during viral replication and phage maturation is described.

Bacteriophage φX174 contains a circular molecule of single-stranded DNA in an icosahedral coat of protein. Synthesis of single-stranded phage DNA involves an intermediate consisting of a double-stranded circular replicative-form (RF) DNA with an extended tail of single-stranded viral DNA ("rolling circle" or sigma [σ] structure) (3, 4, 12, 20, 29).

A particularly interesting feature of φX174 morphogenesis is that production of intact, single-stranded viral DNA depends on the functions of seven phage-coded genes, including those specifying virion structural proteins F (major capsid protein), G (major spike protein), and H (spike protein) and nonvirion proteins A, B, C, and D (2, 5, 7, 18, 21, 32). Whether or not gene J, whose product is a virion protein (D. Freymeyer et al., cited in reference 26), is involved in this process has not been determined. The only known phage gene that is not involved in single-stranded DNA synthesis is gene E (lysis) (22).

Previously we found a DNA-protein complex in cells infected with wild-type phage (and with a mutant of the lysis gene E). This complex has an S value of 50 (50S complex) and is a precursor of mature phage particles (6, 7). The 50S complex contains σ structure DNA having single-stranded viral DNA tails up to one genome in length and phage-coded proteins A, A* (the smaller of the two products of gene A [23, 24]), D, F, G, H, and J and several host proteins.

Two alternative models for viral DNA synthesis in the 50S complex have been proposed (6, 8). In one model, the 50S complex is formed by aggregation of a preformed capsomeric structure with an RFII molecule having a short tail of viral DNA. Viral DNA synthesis continues in the 50S complex, and the growing single-stranded tail is packaged in the capsomeric structure. As one round of viral DNA synthesis nears completion, cleavage of the viral DNA strand occurs, resulting in the release of RFII and a phage precursor particle. In the other model, the 50S complex is formed by successive aggregation of virion proteins with the viral strand of the σ structure DNA. Synthesis of viral DNA may depend on continued addition of viral proteins to the σ-structure DNA. Capsid assembly and viral DNA synthesis proceed until a capsomeric structure is formed, as one round of viral DNA replication nears completion. Subsequent cleavage of the viral DNA results in the formation of a phage precursor and a free RFII molecule.

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Previously we have described the presence of a protein particle from phage-infected cells that is a precursor of mature phage and sediments with an S value of 12 (12S particle) in a sucrose gradient (27, 33). The 12S particle consists of five molecules of the major capsid protein, F, and five molecules of the spike protein, G, and is proposed to be the basic structural unit of each of the 12 vertexes of the icosahedral virion particle. The two models discussed above would predict two different modes for utilization of the 12S particle during phage morphogenesis. If the first model is valid, 12 12S particles could associate to form a capsomeric structure independently of viral DNA replication. The second model would require about one complete round of viral DNA replication to form a capsomeric structure from 12 12S particles.

In this paper we describe results supporting the involvement of a preformed capsomeric structure, assembled in the absence of viral DNA synthesis, during the maturation of ϕX174. We found that such a structure is formed when viral DNA synthesis is inhibited either by thymidine starvation or by mutation in gene A or C. The capsomeric structure seems to be converted to mature phage particles on restoration of viral DNA synthesis.

A model of the morphogenetic pathway of ϕX174 is proposed and discussed.

MATERIALS AND METHODS

Bacteria and bacteriophages. *Escherichia coli* HF4704 (*thyA hcr*) was used as a nonpermissive host for amber and ochre mutants of ϕX174. *E. coli* CR63.1 and *su*20C were permissive hosts for amber and ochre mutants, respectively. Amber mutants of ϕX174 used were H90 (gene A) and N11 (gene E). *och*6 contains an ochre mutation in gene C. *E. coli* *su*20C and *och*6 were given to us by R. L. Sinsheimer, and *E. coli* CR63.1 was from I. Tessman.

Media and buffer. Media and buffer have been described previously (6, 11). Infection and preparation of cell extracts were performed as detailed previously (6). For experiments involving starvation for thymidine, infected cells were collected by centrifugation at room temperature, washed once with HF complete medium (11) without thymidine, and suspended in the washing medium. Low T-HF medium is complete HF medium containing 0.2 μg of thymidine per ml.

Centrifugation. The extracts were centrifuged through a 5 to 30% linear sucrose gradient in EDTA buffer with a 55% CsCl bed at the bottom (6). Centrifugation was performed in an SW41 rotor at 39,000 rpm for 150 min at 5°C (Fig. 1). When better resolution of 114S virion and 108S material was needed, the extracts were centrifuged through 15 to 30% linear sucrose gradients in EDTA buffer in an SW50.1 rotor at 49,000 rpm for 90 min at 5°C (see Fig. 4 and 5).

Gel electrophoresis. Slab gel electrophoresis was performed as described by Studier (30) by using the polyacrylamide gel system in method 2 of Siden and Hayashi (27). Cylindrical gel electrophoresis was described by Gelfand and Hayashi (11).

Electron microscopy. Grids with carbon-coated Formvar film were floated in drops of samples for 1 to 2 min, and the specimens were negatively stained with 0.5% neutral sodium phosphotungstate. Micrographs were taken by a Philips EM 300 electron microscope at 65 kV.

RESULTS

Accumulation of a protein aggregate in the absence of viral DNA synthesis. HF4704 cells infected with an amber A mutant (H90) or an ochre C mutant (*och*6) were labeled with radioactive amino acids. Extracts of the infected cells were prepared and analyzed by sucrose gradient sedimentation. Results of these experiments are shown in Fig. 1a and b. Neither progeny RF nor viral DNA synthesis occurs in nonpermissive cells (HF4704) infected with phage containing an amber mutation in gene A (5, 18, 31), but all phage-coded proteins (except the gene A proteins) are synthesized (11). Extracts of these infected cells contained material sedimenting at an S value of 108 (Fig. 1a; the S value was determined by using phage particles [114S] as a marker in a separate gradient).

In C mutant-infected cells in which viral DNA synthesis is extremely reduced, radioactive material sedimenting with an S value of 108 was also found (Fig. 1b). As described in a previous paper (8), a small amount of radioactivity was incorporated into the phage peak probably due to leakiness of the ochre mutation (10). In cells infected with B, D, F, or G mutants, no 108S material was found (8). In a separate experiment, HF4704 cells were infected with a gene E mutant (lysis mutant, N11), and replication of RF DNA was allowed to proceed. When viral DNA synthesis started, thymidine was eliminated from the medium by washing the infected cells and suspending them in complete medium without thymidine. During the thymidine starvation period, the infected culture was labeled with radioactive amino acids, and an extract of the infected cells was prepared. Analysis of the extract by sucrose gradient sedimentation revealed a radioactive peak with an S value of 108 (Fig. 1c).

We have previously shown that 108S material isolated from cells infected with gene C mutant does not contain DNA (8). The 108S material from cells infected with the gene A mutant should not contain viral DNA because no phage DNA replication other than parental RF DNA synthesis occurs in these cells. When

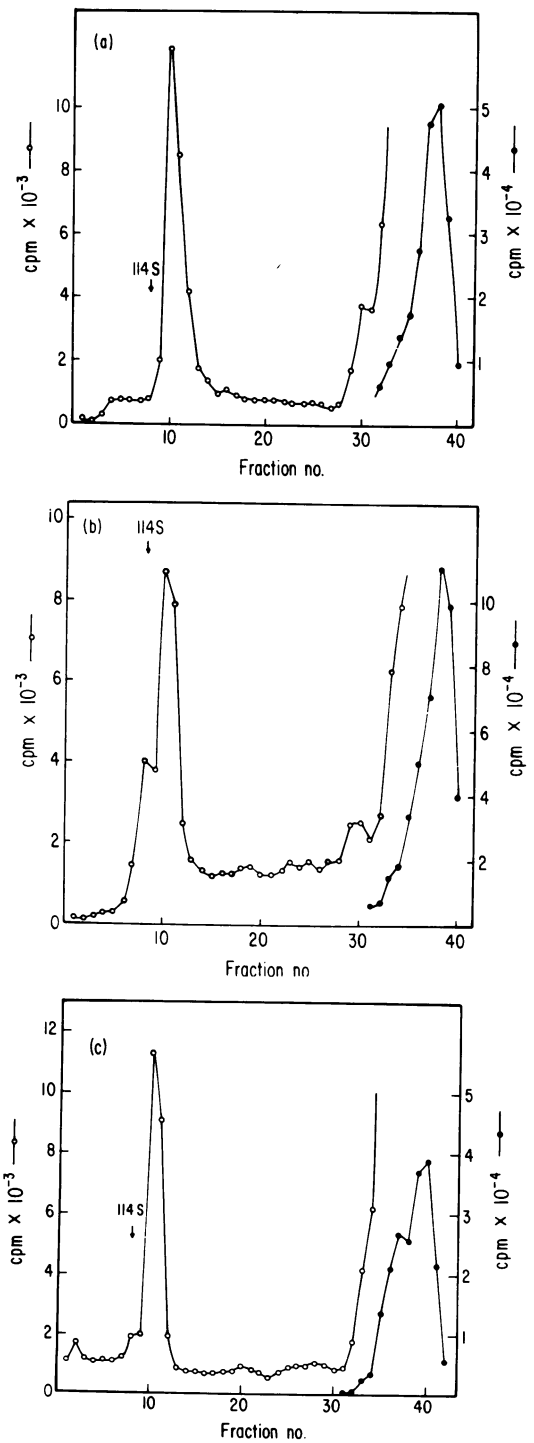

FIG. 1. *Existence of 108S material in phage-infected cells. Portions of a culture of E. coli HF4704 in HF complete medium were treated with mitomycin C to reduce host DNA synthesis (21), infected with an amber mutant of gene A (H90) or an ochre mutant of gene C (och6), and labeled with [¹⁴C]lysine (0.2 μCi/ml and 1 μg/ml) between 12 and 22 min postinfection. Another portion of culture was infected with an amber mutant of lysis gene E (N11) in low T-HF complete medium and washed at 12 min postinfection with HF medium without thymidine to remove thymidine. After being washed the cells were suspended and incubated in HF medium without thymidine. The culture was labeled with [¹⁴C]lysine (0.2 μCi/ml and 1 μg/ml) between 20 and 30 min after resuspension in medium without thymidine. At the end of the labeling period, cells were collected and extracts were prepared and sedimented through linear 5 to 30% sucrose gradients in an SW41 rotor at 39,000 rpm for 150 min at 5°C. [¹⁴C]lysine-labeled phage was centrifuged in a separate sucrose gradient, and its position (114S) is indicated by the arrows. (a) H90-infected cells; (b) och6-infected cells; and (c) N11-infected cells under thymidine starvation conditions.*

DNA in cells infected with the gene E mutant was labeled between 3 and 10 min postinfection and then the infected cells were starved of thymidine for 20 min, no radioactive DNA was found in 108S materials accumulated during thymidine starvation (data not shown).

Protein composition of 108S material. The 108S material from the sucrose gradients (Fig. 1a, b, and c) were analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels to determine their protein composition. 132S particles that had the same protein composition as the 114S phage particles (F, G, H, and J) except for the additional presence of D protein (34) were used as reference markers for electrophoresis. 108S materials isolated from cells infected with gene A, C, and E mutants under thymidine starvation conditions contained F, G, H, and D proteins but lacked J protein (Fig. 2). Quantification of an electropherogram of cylindrical polyacrylamide gels indicated that the relative amounts of F, G, H, and D proteins in 108S materials were very similar to those in the 132S particle (Table 1) and to those in the 50S complex (6).

Electron micrographs of 108S materials. 108S materials found under the three conditions detailed in Fig. 1 were isolated by sedimentations in sucrose gradients and examined by electron microscopy. For comparison, 132S particles and 114S mature phages were also examined. Electron micrographs are shown in Fig. 3. 108S materials from all three sources are identical in structure, and their dimensions are very similar to those of the 132S particle. Both 108S material and the 132S particle are spherical in shape and are distinguishable from the phage particle, which has a smaller diameter and spikelike projections. The phosphotungs-

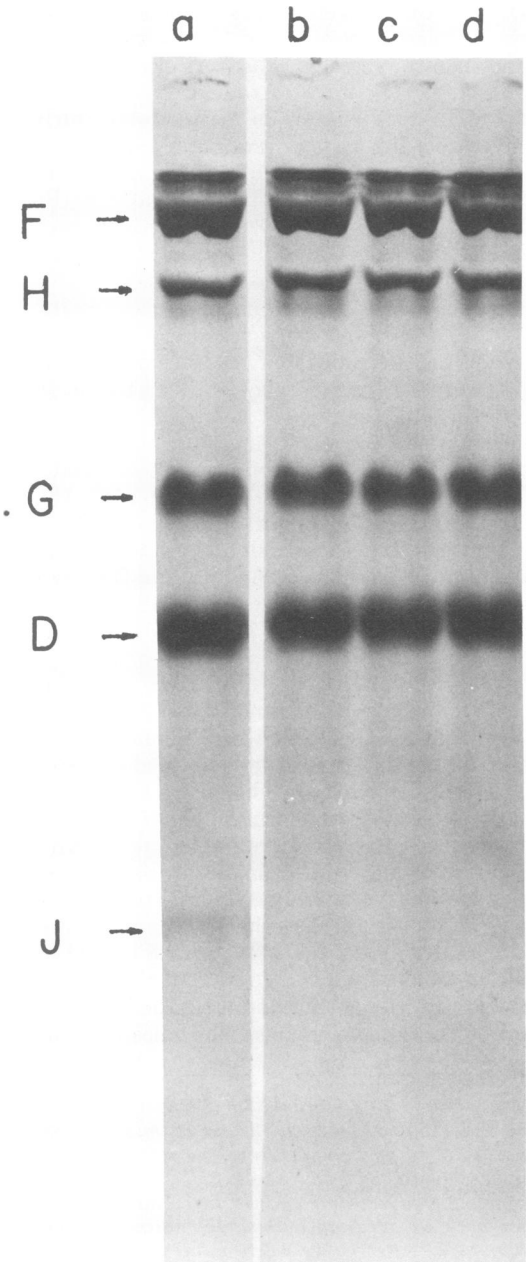

FIG. 2. *Autoradiograms of sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis of 108S material. 108S peak fractions (fraction 10) from each of the gradients in Fig. 1 were analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide slab gel as described in the text. 132S particles labeled with [^{14}C]lysine were prepared from N11-infected cells as described previously (6) and used as standards. (a) 132S particle; (b) 108S material from H90-infected cells; (c) 108S material from och6-infected cells; and (d) 108S material from N11-infected cells under thymidine starvation conditions. Positions of F, H, G, D, and J proteins in the gel are shown by arrows.*

tate stain seems to penetrate the 108S material but not the 132S particle or the 114S phage particle, suggesting that the 108S material has an empty shell structure.

Fate of accumulated 108S material on resumption of viral DNA synthesis. HF4704 cells were infected with a lysis mutant of ϕX174 (N11) in complete medium containing thymidine and were allowed to accumulate RF molecules. When single-stranded viral DNA synthesis began, infected cells were washed and suspended in complete medium without thymidine to starve them of thymidine. During the thymidine starvation period, [^{3}H]lysine was added to the culture for 3 min. Then an excess amount of unlabeled lysine was added to the culture, which was divided into two parts. One part was incubated in the continued absence of thymidine, and samples were taken after adding unlabeled lysine at specified times. The other part was supplemented with thymidine, and samples were collected at specified times after adding thymidine. Extracts were prepared from these cells and analyzed in sucrose density gradients (Fig. 4). In cells that had been pulse-labeled with [^{3}H]lysine and chased with unlabeled lysine in the absence of thymidine, 108S material was formed (Fig. 4a) and did not disappear even after prolonged incubation (Fig.

TABLE 1. *Protein composition of 108S material, 132S particle, and 114S mature phage*[a]

Protein	108S(A)[b]	108S(C)[c]	108S(E)[d]	132S	114S
F	1.00	1.00	1.00	1.00	1.00
H	0.21	0.20	0.20	0.18	0.18
G	0.38	0.36	0.36	0.37	0.36
D	1.10	1.12	1.08	1.05	<0.03
J	0	0	0	0.20	0.20

[a] 108S peak fractions (fraction 10) from each of the gradients in Fig. 1 were analyzed by cylindrical sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described by Gelfand and Hayashi (11). Gels were sliced, solubilized, and counted in a scintillation counter. 132S particle and 114S mature phage were isolated from cells infected with mutant N11 and labeled with[^{14}C]lysine between 10 to 20 min after infection as described previously (6). Distribution of radioactivity is expressed in relative amounts with the radioactivity of F as 1.00.

[b] 108S material isolated from H90-infected cells.

[c] 108S material isolated from *och*6-infected cells.

[d] 108S material isolated from N11-infected cells under thymidine starvation conditions.

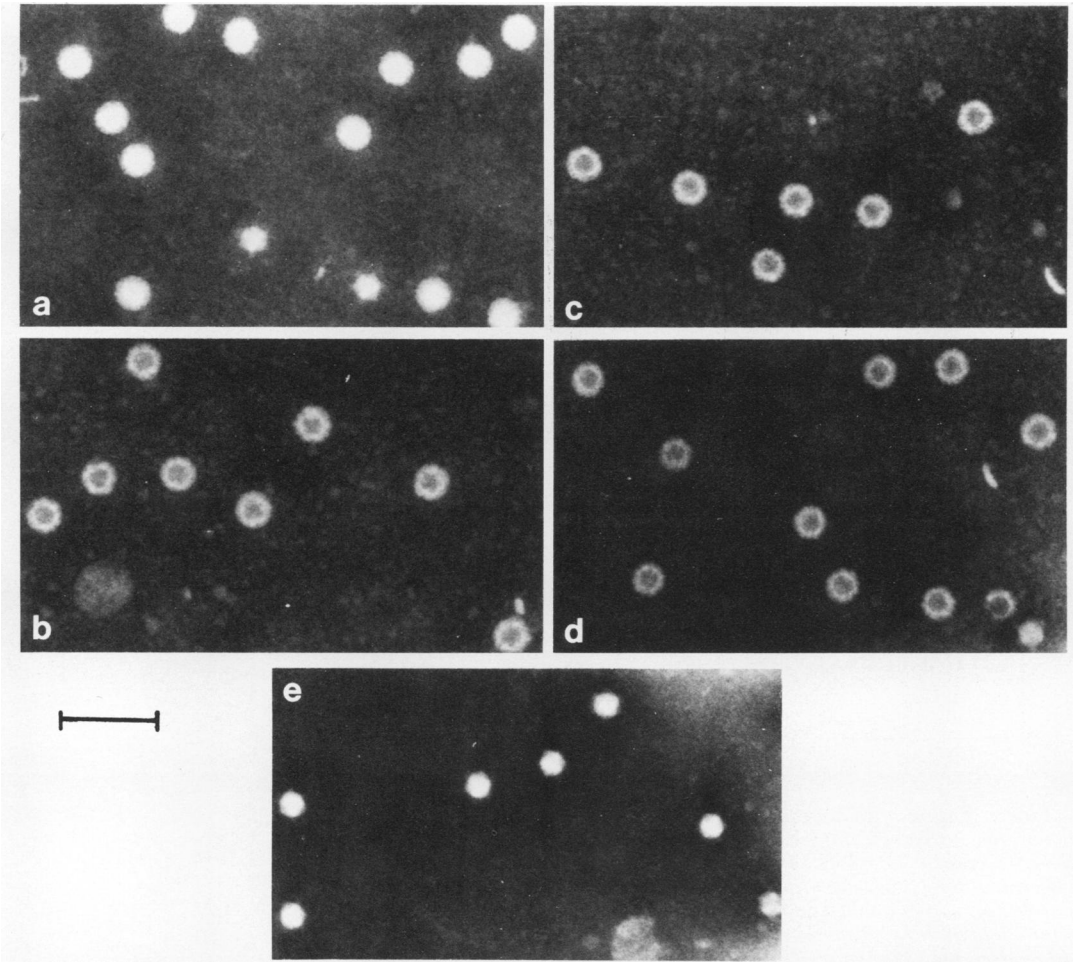

FIG. 3. *Electron micrographs of 132S particle, 114S phage particle, and 108S material. (a) 132S particle (114S particles existing in this preparation are seen); (b) 108S material from H90-infected cells; (c) 108S material from och6-infected cells; (d) 108S material from N11-infected cells under thymidine starvation conditions; and (e) 114S particle. Bar, 100 nm.*

4b). However, when thymidine was added to the culture, 108S material that had been labeled during thymidine starvation gradually disappeared with a concomitant increase of counts in the 114S and 132S particles (Fig. 4c and d).

The rate of disappearance of accumulated 108S material after addition of thymidine to infected cells that previously had been starved of thymidine was quite slow. During the normal infection process, utilization of 108S material appears to be very rapid, since no such material is observed even after a short pulse label. This raised the possibility that infected cells starved of thymidine might be altered in their ability to assemble phage particles after restoration of the thymidine. This possibility was tested by an experiment similar to that described in the legend to Fig. 4, except that the pulse and pulse-chase with [^{3}H]lysine were performed after restoration of thymidine. Cells infected with a lysis mutant (N11) were allowed to accumulate RF molecules and then were deprived of thymidine for 15 min. Thymidine was then added to the culture. Five minutes after restoration of thymidine, the cells were pulse-labeled for 2 min with [^{3}H]lysine and chased with an excess amount of unlabeled lysine. Portions of the culture were removed at the specified times after adding unlabeled lysine. Cell extracts were prepared and analyzed by sucrose gradient centrifugation (Fig. 5). Significant

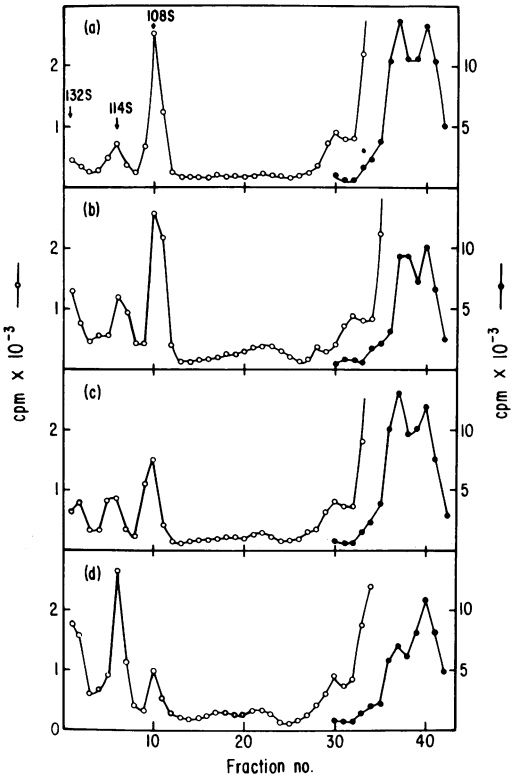

FIG. 4. *Fate of 108S material accumulated during thymidine starvation after restoration of thymidine. HF4704 cells in low T-HF complete medium were infected with N11. Twelve minutes after infection, the infected cells were washed and suspended in medium without thymidine. Ten minutes later, the culture was pulsed for 3 min with [³H]lysine (10 μCi/ml and 1 μg/ml) and then chased with unlabeled lysine (250 μg/ml). This culture was divided into two parts. One was incubated in the continued absence of thymidine, and samples were taken 7 (a) and 30 min (b) after adding unlabeled lysine. The other part of the culture was supplemented with thymidine (5 μg/ml) that was added 7 min after initiation of the chase, and samples were removed 10 (c) and 30 min (d) after the chase. Extracts were prepared and analyzed by sedimentation through sucrose gradients (15 to 30% as described in the text) in an SW50.1 rotor at 49,000 rpm for 90 min at 5°C. ¹⁴C-labeled 114S phage particles were used as a sedimentation marker.*

amounts of radioactivity were found in the 108S peak immediately after pulse labeling (5 to 7 min after thymidine restoration, Fig. 5a) and even after a 5-min chase in the presence of unlabeled lysine (12 min after thymidine restoration, Fig. 5b). After a prolonged chase, the 108S material slowly disappeared, with a corresponding increase of radioactivity in the 114 and 132S peaks (Fig. 5c).

DISCUSSION

We have isolated 108S material from three different sources; (i) amber A mutant-infected cells in which parental RF (RF-containing incoming viral strand DNA) is formed but neither progeny RF nor single-stranded viral DNA is synthesized (5, 18, 31); (ii) cells infected with an ochre mutant in gene C whose product is required for synthesis of single-stranded viral DNA (8, 10); or (iii) cells infected with a lysis mutant and starved for thymidine during the period of single-stranded viral DNA synthesis. The 108S materials made under these three conditions have identical structures and physically resemble the spherical 132S particle when ex-

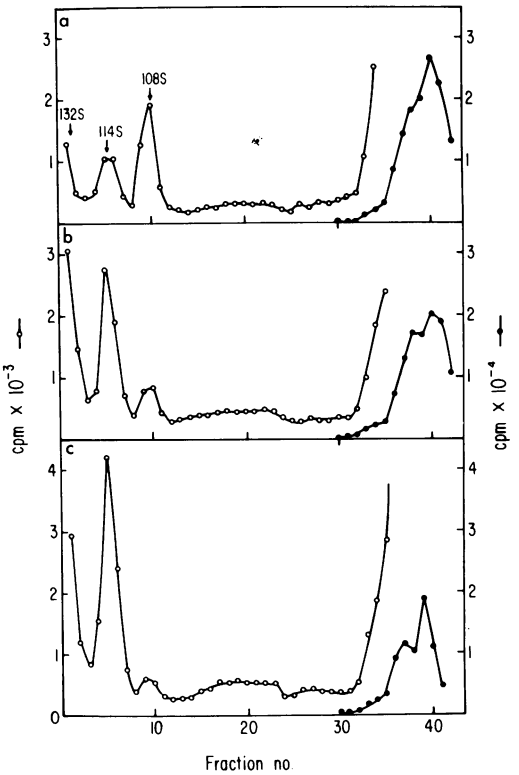

FIG. 5. *Sedimentation analysis of 108S material formed in the presence of thymidine in phage-infected cells previously starved of thymidine. HF4704 cells were infected with N11 and starved of thymidine as described in the legent to Fig. 1. After 15 min of starvation, thymidine was added to the culture (5 μg/ml). The culture was labeled with [³H]lysine between 5 and 7 min after restoration of thymidine and chased with unlabeled lysine (250 μg/ml). Samples were removed after chasing for 5 (a), 10 (b), and 20 min (c). Preparation of extracts and analysis by sucrose gradient centrifugation were as described in the legend to Fig. 4.*

amined under an electron microscope except that stain readily penetrates into 108S material but not into the 132S particle (Fig. 3). 108S material does not contain DNA. The protein composition of 108S material is characterized by the presence of F, G, H, and D proteins in the same proportion as those found in the 132S particle (Table 1). These observations indicate that 108S material isolated from these three sources is identical and resembles the capsid structure of the 132S particle. Our 132S particle is identical to the infectious 140S particle described by Weisbeek and Sinsheimer (34). The 132S particle can be converted in vitro to the 114S phage particle and, except for the additional presence of D protein on the 132S particle, the composition of the 132 and 114S particles is identical (Table 1; 9, 34). 108S material formed during thymidine starvation in cells infected with lysis mutants was stable and maintained its structure after prolonged thymidine starvation. After release from thymidine starvation, the 108S material was converted to 132S and 114S virion particles (Fig. 4). This would indicate that the 108S material is a precursor for phage. The rate of conversion of 108S to phage after adding thymidine to infected cell cultures previously starved of thymidine was slow and conflicted with the very rapid rate at which the 108S material is probably converted to phage under normal infection conditions. It appears that thymidine starvation altered the ability of infected cells to support phage maturation after restoration of thymidine (Fig. 5). It is possible that some precursor of phage morphogenesis becomes deficient during thymidine starvation and must be replenished after adding thymidine. We have observed that removing thymidine from a culture of phage-infected cells that had been prelabeled with radioactive thymidine led to conversion of RFII to RFI (H. Fujisawa and M. Hayashi, unpublished data).

The molecular structure and kinetics of utilization of the 108S material suggest that it is a capsomeric precursor of phage particles. We have not been able to observe the conversion of 108S material to the 50S complex. As we described in a previous paper (6), formation of phage from 50S complex is very rapid and would be difficult to detect by the present method.

The 108S material formed during thymidine starvation of cells infected with a lysis mutant seemed to be stable (Fig. 4), but the possibility exists that stability of 108S material is altered after adding thymidine. If this were the case, 108S material accumulated during thymidine starvation could conceivably dissociate after restoration of thymidine to the culture. The dissociated protein components could subsequently be utilized to produce phage particles. To distinguish this possibility, density labeling of 108S material in cells infected with a lysis mutant was performed by using [^{15}]N H_4Cl during thymidine starvation followed by a chase with [^{14}]N H_4Cl after adding thymidine. Demonstration of density transfer from 108S material to the mature virion has not been successful mainly due to density labeling of DNA precursors. However, the almost stoichiometric relationship between disappearance of 108S material and appearance of 114S and 132S particles (Fig. 4a, c, and d) tends to reject dissociation of 108S material to its components because unlabeled phage proteins would be synthesized during the chase with unlabeled lysine, resulting in dilution of the radioactivity of virion precursor proteins.

A proposed working hypothesis on the roles of several gene products of φX174 during the phage maturation process is presented schematically in Fig. 6. A description of the features of the model is in the figure legend.

A number of experimental observations support the present working hypothesis. Tonegawa and Hayashi (33) showed that 6S, 9S, and 12S particles are precursors of phage particles. The 6S particle is a pentamer of G protein, and the 9S particle is a pentamer of F protein. Siden and Hayashi (27) determined that the catalytic activity of gene B protein allows 6 and 9S particles to aggregate, forming the 12S particle, which is postulated to be the structural unit of each of the 12 vertexes of the icosahedral virion. In the present paper, we have shown that a 108S capsomeric structure that is a phage precursor was synthesized independently of DNA replication. We propose that assembly of the 108S complex is the result of aggregation of 12 of the 12S particles with the active participation of the gene D product as a scaffolding protein. This role of gene D protein in capsid assembly is supported by the fact that the 12S particle, but not the 108S material, is formed under restrictive conditions in cells infected with gene D mutants (8, 33), and the gene D protein is associated with several intermediates (108S material, 50S complex, 132S [140S] particles) involved in phage maturation (6, 9, 34). We have shown that 132S particles are not efficiently inactivated by antiserum against 114S phage particles (8). When 132S particles are first converted to 114S particles in vitro, the converted 114S particles are neutralized by the antiserum to the same extent as 114S phage particles. This indicates that D protein protects phage antigenic determinants in the 132S particle. The similarity in appearance between 108S

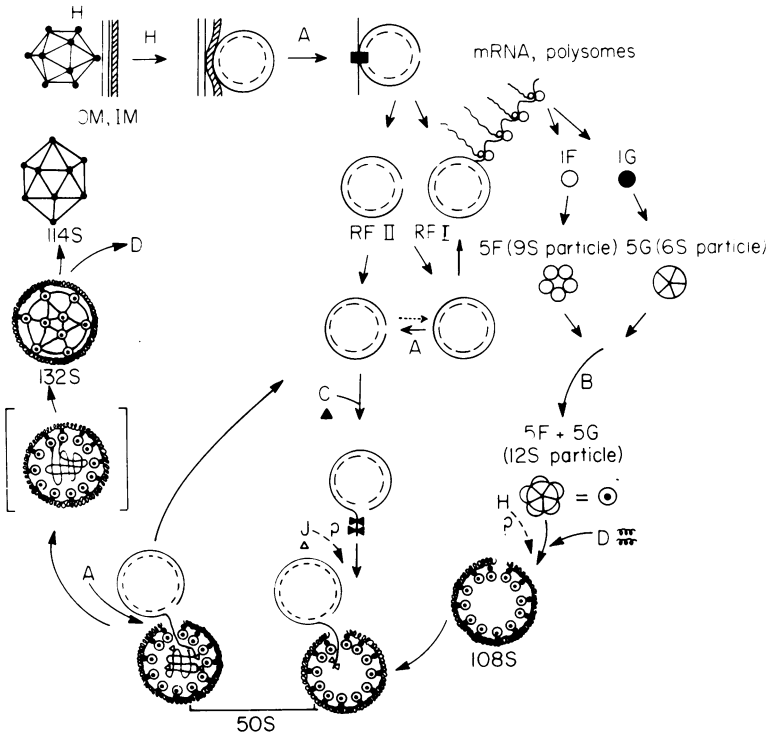

FIG. 6. *Model for morphogenesis of bacteriophage φX174. Five molecules of the major capsid protein F combine to form the 9S particle, and five molecules of the spike protein G associate to form the 6S particle. The catalytic activity of the gene B protein results in the aggregation of a 9S particle and a 6S particle forming the 12S particle. The assembly of 12 of the 12S particles into the 108S capsomeric structure requires the scaffolding function of the gene D protein. The steps leading to the formation of the 108S structure occur independently of phage DNA replication. RFII molecules, which are templates for single-stranded viral DNA synthesis, accumulate during the early stages of the infection process, either directly as a result of RF replication or from RFI by the endonucleolytic activity of gene A protein(s). The initial stages of viral DNA replication involve RFII and the gene C protein and result in the formation of RFII molecules that can associate with the 108S capsomeric structure to give rise to the 50S complex. Continuation of viral DNA synthesis by way of a σ-structure-replicating intermediate and packaging of the single-stranded DNA into the capsomeric structure take place in the 50S complex. As one round of viral DNA replication nears or reaches completion, the gene A protein(s) cleaves the single-stranded DNA tail of the σ structure, resulting in the separation of RFII from a phage precursor particle. The genome of single-stranded DNA in this precursor particle is circularized instantaneously at the time of cleavage or very soon afterward to form the 132S particle that can, at least in vitro, be converted to the mature 114S virion of φX174. This figure includes the pilot protein model of H protein proposed by Jazwinski et al. (19). Puga and Tessman (25) and Hayashi and Hayashi (15) showed that RFI form is the template for transcription in vivo, and Hayashi and Hayashi (16) showed that RNA polymerase has a stronger affinity to RFI than to RFII in vitro. This information is also incorporated in this figure. Further details of the model and experimental results supporting its validity are discussed in the text. OM, Outer membrane; IM, inner membrane; -----, minus strand; ——, plus strand.*

material and the 132S particle by electron microscopy suggests that association of D protein with these two structures occurred in an identical manner (Fig. 3). Several observations indicate that the presence of D protein on the 132S particle is not due to a fortuitous aggregation of D protein with 114S phage particles. The addition of 114S phage particles to infected-cell extracts containing D protein does not lead to their conversion to 132S particles (34). Exposure of 132S particles to buffers of low ionic strength without divalent cations causes formation of defective particles, whereas 114S phage particles are not altered by such treatment (34). 114S phage particles are not affected by Zn^{2+}, but 132S particles are inactivated by its presence (9). These observations showing greater stability of the 114S phage particle compared to the 132S particle imply an alteration or rearrangement of capsid conformation on removal

of D protein from the 132S particle in vitro to form the 114S phage particle. This conversion is apparently irreversible. By inference we propose that the presence of D protein in the 108S capsomeric structure is not artifactual and is required to assemble and/or maintain the structural conformation of the phage capsid during morphogenesis.

The synthesis of intact single-stranded viral DNA is tightly coupled to the phage morphogenetic pathway. This coupling can be explained by our previous finding that σ-structure DNA, which is the intermediate for viral DNA replication, is associated with phage proteins A, A*, D, F, G, H, and J and with several host proteins in a DNA-protein complex that has an S value of 50 (6). The 50S complex is a precursor of the mature phage and contains D, F, G, H, and J proteins in the same proportions as those found in the 132S particle. The present model proposes that the 50S complex is formed by the association of the 108S capsomeric structure with an RFII molecule. "Rolling-circle" synthesis of viral strand DNA and subsequent packaging of viral DNA in the capsomeric structure takes place in the 50S complex.

We have previously suggested that the gene C protein is responsible for maintaining the availability of RFII for formation of the 50S complex (8). In the absence of a functional gene C, newly synthesized RFII is converted rapidly to RFI (or RFI*, which is RFI complexed with RNA [13-15]). This rapid conversion of RFII to RFI does not occur in cells infected with a mutant of genes B, D, F, or G (8). The deficit of RFII capable of 50S complex formation in cells infected with a gene C mutant prevents viral DNA synthesis in spite of the presence of 108S capsomeric structures. The mechanism by which the gene C protein maintains RFII for utilization as template during viral DNA synthesis is not known. We have speculated that the gene C protein has DNA-binding properties and functions by attaching to RFII to prevent its conversion to RFI (8). No evidence exists to favor this or any other mechanism for the action of gene C protein.

In a previous paper (7), we reported that the 50S complex is formed in cells infected with a temperature-sensitive mutant of gene A at either the permissive or restrictive temperature. The 50S complex formed at the permissive temperature is indistinguishable from the 50S complex formed in cells infected with wild-type phage and contains σ-structure DNA having single-stranded tails of various sizes up to 1 genome in length. When cells infected at the permissive temperature with the temperature-sensitive mutant of gene A are brought to the restrictive temperature, thymidine incorporation ceases abruptly, and the 50S complex accumulates. The 50S complex accumulated under these conditions contains σ-structure DNA with homogeneous single-stranded tails that are 1 full genome in length. On shift-down to the permissive temperature, the 50S complex accumulated at the restrictive temperature is converted to mature phage particles. These observations indicate that the gene A product(s) cleaves unit-genome-size viral DNA from the σ structure in the 50S complex. In the absence of cleavage of the single-stranded tail, viral DNA replication stops after completing one round of synthesis in the 50S complex.

Cleavage of a unit length of viral DNA by the gene A protein(s) produces an RFII molecule and a phage precursor particle. The RFII can be recycled into further rounds of viral DNA synthesis by way of the RF pool and the 50S complex. The phage precursor released from the 50S complex may be expected to contain linear DNA enclosed in a capsomeric structure. Such an intermediate has not been observed, suggesting that ligation of the ends of the linear, single-stranded DNA may be a very rapid process, perhaps occurring instantaneously at the time of cleavage. Denhardt (1) has proposed a model involving a mechanism of strand translocation by which cleavage and ligation of a unit length of viral DNA occur simultaneously.

Circularization of viral DNA gives rise to the 132S particle. The exact in vivo relationship between the 132S particle and the 114S phage particle is not clear. The 132S particle can be converted very efficiently in vitro to 114S phage particles under the appropriate conditions. Neither Weisbeek and Sinsheimer (34) nor we have been able to demonstrate conversion of 132S particles to 114S phage particles in vivo (9). In fact, pulse-chase experiments indicate that these two particles are formed simultaneously in phage-infected cells with no apparent precursor-product relationship between them (8, 9). It is possible that the 132S particle and the 114S phage particle are formed by two separate pathways branching from a common precursor (such as the 50S complex). Alternatively, the 132S particle could possibly be the final intracellular product of φX174 morphogenesis, and conversion to the 114S phage particle could occur only after lysis and subsequent purification procedures.

Our model proposes possible functions for the products of φX174 genes A, B, C, D, F, and G during viral DNA replication and phage maturation. The roles of genes H and J are not clear. Jazwinski et al. (19) have proposed that the gene H product functions as a pilot protein that

directs the incoming viral DNA to the DNA replication site(s) on the host membrane during the early stages of phage infection. Siegel and Hayashi (28) observed two kinds of defective particles in cells infected with mutants of gene H. One (110S particle) contains infectious single-stranded DNA complexes with F, G, and J proteins. The other (70S particle) contains linear single-stranded DNA shorter than the unit length associated with F, G, and J proteins. It is possible that phage assembly in the absence of functional gene H protein leads to a defective particle whose DNA is easily attacked by nuclease, resulting in particles with degraded single-stranded DNA (18). Alternatively, a deficiency of gene H protein could increase the frequency of aberrant termination of viral DNA synthesis before completion of one round of replication. Our observation that the 110S particle is not a precursor of the 70S particle (K. Spindler and M. Hayashi, unpublished data) supports this possibility. In cells infected with wild-type phage, the 50S complex and the 108S material contain H protein. Therefore, H protein must enter into the proposed pathway somewhere between the 12S particle and the 108S material. We have identified a particle that is identical to the 12S particle except for the presence of a small amount of H proteins (unpublished data). Whether or not this particle is a precursor for mature phage is not clear.

Another gene product whose function during the maturation process is unknown is J protein. Because of the unavailability of mutants in this gene, no function has been assigned to gene J. The 50S complex, but not 108S material, contains J protein. We speculate that J protein enters into the proposed pathway somewhere between RFII and the 50S complex. J protein is preferentially labeled with lysine, arginine, or glycine (E. Linney, personal communication), which may indicate its DNA-binding (or condensing) characteristics.

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