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Functions of Gene C and Gene D Products of Bacteriophage φX174

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Phage-related materials existing in cells infected with various mutants of bacteriophage φX174 were investigated. A novel species of replicative-form (RF) DNA was found in cells infected with a phage mutant of gene B, C, D, F, or G. This species, called RFI*, sedimented at a position between RFI and RFII in a neutral sucrose gradient. It was converted to RFI upon denaturation in alkali, denaturation in formamide and subsequent renaturation, or RNase treatment at low ionic strength. In cells infected with a phage mutant of gene C, RFI* was derived from pulse-labeled RFII after a short chase. The conversion of pulse-labeled RFII to RFI* was not observed in cells infected with a mutant of gene B, D, or F. A possible function of the C gene product of φX174 could be to prevent the conversion of RFII to RFI, thereby maintaining the availability of RFII to act as the template for single-stranded viral DNA synthesis. A protein complex containing no DNA, which sedimented with an *S* value of 108 in a sucrose gradient and contained virion proteins F, G, and H, and nonvirion protein D, was found in cells infected with the gene C mutant. A possible function of protein D was considered as a scaffolding protein for assembly of phage structural proteins.

Bacteriophage φX174 contains a single-stranded circular molecule of DNA in an icosahedral coat. In the early stage of the infection process, double-stranded circular DNA (replicative-form [RF] DNA) is synthesized predominantly. Gene A is the only phage gene required for the replication of RF DNA (9, 22, 23, 37). Viral DNA synthesis occurs at the late stage of infection and requires at least six phage-coded genes: A, B, C, D, F, and G (5, 23, 26, 27). The gene A product is necessary for cleavage of a unit length of the viral DNA from the RF DNA with growing viral strand DNA tail (11). Gene B controls the formation of an intermediate protein particle that sediments with an *S* value of 12 (12*S* particle [34, 38]). The 12*S* particle is composed of a pentamer of major capsid protein F and a pentamer of spike protein G. The B gene product catalyzes the aggregation of F pentamer (9*S*) and G pentamer (6*S*) to form the 12*S* particle. Without 12*S* particle formation, no viral DNA synthesis is observed. Functions of gene C and gene D during viral DNA synthesis still remain to be elucidated. Funk and Sinsheimer (12) showed that formation of progeny phage was delayed in cells infected with an ochre mutant of gene C. They interpreted this result to be due to leakiness of suppression of the ochre mutation and suggested that only a small amount of gene C product is required for phage maturation. They also observed the accumulation of an RF molecule that sedimented in a preformed CsCl sedimentation gradient at the same position as RF molecules with single-stranded tails, which are found in cells infected with wild-type phage.

In a previous paper (10) we showed that a complex sedimenting with an *S* value of 50 in a sucrose gradient (50*S* complex) is an intermediate in the production of mature phage. The 50*S* complex contains an RF molecule with a single-stranded tail up to one genome in length (σ structure [6]). This strucuture has been shown to be an intermediate in the synthesis of single-stranded viral DNA (7, 14, 24, 36). In the 50*S* complex, the σ structure is associated with viral-coded proteins A, A* (a smaller gene A product [28]) D, F, G, H, and X (a phage structural protein, whose origin is uncertain) and several host proteins. If the σ structure accumulates in cells infected with C mutants as reported by Funk and Sinsheimer (12), it may be interesting to examine the DNA protein complexes containing the σ structure in these infected cells. Also, since the 50*S* complex contains D protein, formation of the 50*S* complex may be prevented in infected cells deficient in D protein.

In this paper, we describe the results of the analyses of phage-related complexes found in

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cells infected with mutants defective in single-stranded viral DNA synthesis and discuss possible roles of genes C and D in the assembly process of ϕX174.

MATERIALS AND METHODS

Bacteria and bacteriophages. *Escherichia coli* HF4704 was used as a nonpermissive host for amber and ochre mutants. *E. coli* CR63.1 and *E. coli* su20C were permissive hosts for amber and ochre mutants, respectively. Amber mutants of ϕX174 used were H81 (gene D), N11 (gene E), H210 (gene B), and H57 (gene F). An ochre mutant (oc6), which has a mutation in gene C, was used. *E. coli* su20C and oc6 were given to us by R. L. Sinsheimer.

Media and buffer. HF complete medium and HF basal medium were described previously by Gelfand and Hayashi (13). For experiments involving labeling with [^{3}H]thymidine, the thymidine content of HF complete medium was decreased to 0.5 μg/ml. EDTA buffer was 0.1 M NaCl-10 mM sodium phosphate buffer-5 mM EDTA (pH 7.4).

Infection and preparation of the cell extracts. *E. coli* HF4704 was grown to a density of 5×10^8 cells per ml in HF complete medium at 37°C. Cells were treated with mitomycin C (50 μg/ml) for 20 min in the dark without aeration to reduce host DNA synthesis (26). After mitomycin treatment the cells were sedimented, washed once with HF basal medium, and suspended in 0.1 volume of HF basal medium. Cells were then infected with phage at a multiplicity of infection of 7. After a 10-min adsorption period at 37°C, prewarmed HF complete medium (37°C) containing 0.5 μg of thymidine per ml was added to begin the infection. Infected cells were kept at 37°C. At the desired time, the infection was stopped by pouring the culture over 0.5 volume of frozen HF basal medium containing 10 mM NaCN. The cells were sedimented, suspended in 0.1 volume of EDTA buffer containing 200 μg of lysozyme per ml, and incubated for 30 min at 0°C. A few drops of chloroform and $MgCl_2$ (final concentration, 5 mM) were added to the incubation mixture. After 1 min of warming to 37°C, the mixture was allowed to stand for 30 min at 0°C. Extracts were prepared by centrifuging the suspensions at 12,000 rpm for 20 min at 4°C.

Centrifugation. The extracts were centrifuged through a 5 to 30% linear sucrose gradient in EDTA buffer, with a 55% CsCl bed at the bottom in an SW41 rotor (at 39,000 rpm), for 150 min at 5°C. Fractions were collected into polypropylene tubes. Portions of samples were spotted onto filter paper, and radioactivity was determined after a wash with cold 5% trichloroacetic acid-95% ethanol (35).

For further analysis of [^{3}H]DNA, the collected fractions were treated with 0.2% sodium dodecyl sulfate (SDS)-5 mM EDTA at 65°C for 15 min and incubated with 1 mg of Pronase per ml overnight at 30°C.

For neutral sedimentation analysis, [^{3}H]DNA was centrifuged through a 5 to 20% linear sucrose gradient in 5 mM EDTA-3 mM NaOH (pH 10.0) (35) in an SW50.1 rotor at 49,000 rpm for 180 min at 20°C and, for alkaline sedimentation analysis, through a 5 to 20% linear sucrose gradient in 0.2 M NaOH-5 mM EDTA in an SW50.1 rotor at 49,000 rpm for 90 min at 20°C. A mixture of [^{14}C]thymidine-labeled RFI and RFII was added as markers. RFII was prepared from RFI by introducing a single-stranded break with pancreatic DNase (21). After centrifugation, fractions were collected in scintillation vials or in polypropylene tubes. Radioactivity was then determined by mixing with Triton X-100 scintillation fluid as described by Noll (29).

S1 endonuclease digestion. S1 endonuclease has been well characterized as an endonuclease specific for single-stranded DNA (2). S1 nuclease digestion was performed as follows. Appropriate fractions from neutral sucrose gradients were mixed with an equal volume of enzyme solution containing 2×10^3 U of S1 endonuclease per ml in 0.1 M sodium acetate (pH 5.0) and incubated at 37°C for 45 min. Sedimentation analysis of S1 nuclease-treated [^{3}H]DNA was performed as described above.

Gel electrophoresis. Acrylamide gel electrophoresis of proteins was performed by method 2 of Siden and Hayashi (34).

RESULTS

Sedimentation profiles of extracts prepared from cells infected with mutants and labeled with [^{3}H]thymidine. Mitomycin C-treated HF4704 cells were infected with mutants and labeled with [^{3}H]thymidine between 11 and 12 min after infection. The label was chased at 12 min after infection by adding an excess amount of unlabeled thymidine, and thymine and samples were taken 0.5 and 4 min after the chase started. Extracts were prepared and sedimented through sucrose gradients as described above.

As shown previously (10), radioactive counts in cells infected with an E mutant (and with wild-type phage) were incorporated mainly into RFI (supercoiled closed circular RF), RFII (open circular RF with at least one nick in either strand), and the 50*S* complex after a 0.5-min chase (Fig. 1a) and shifted to peaks at 132*S* (mature phage and gene D protein complex [39]), 114*S* (mature phage), and 70*S* after a 4-min chase (Fig. 1b). In cells infected with a C mutant, the radioactive counts were distributed near the top of the gradient (RFI and RFII regions of E mutant-infected cells) and around the 40*S* region immediately after the chase (Fig. 1e) and moved to 114*S* and broader peaks between 40*S* and 60*S* after the 4-min chase (Fig. 1f). When the identical pulse and pulse-chase experiment was performed with cells infected with B, D, or F mutants, distribution of the radioactivity after a short chase (Fig. 1c, g, and i) was very similar to that of C mutant-infected cells, but a longer chase did not change

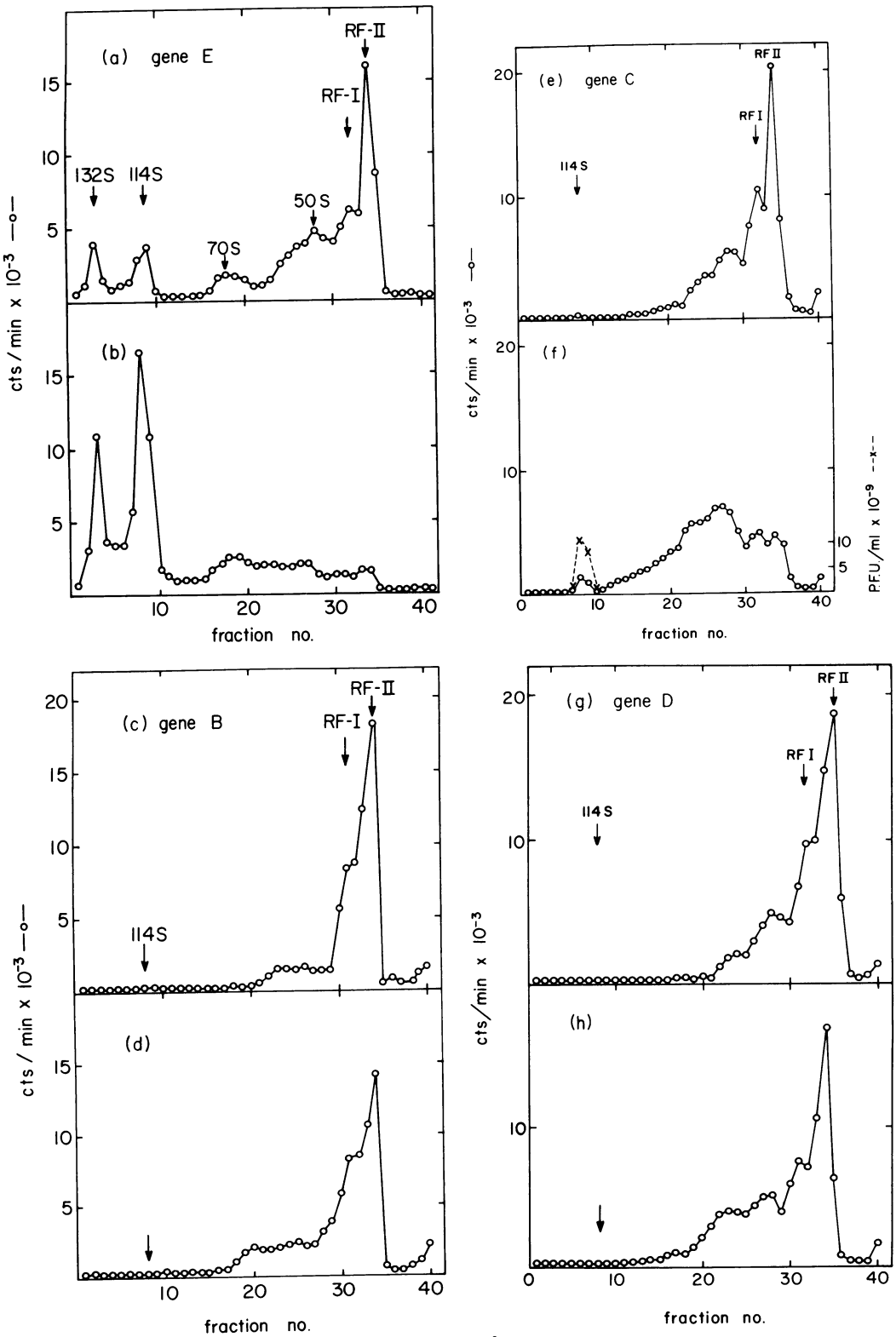
(a) gene E
RF-II
RF-I
132S
114S
50S
70S
(b)
cts / min x 10⁻³ —o—
fraction no.
(e) gene C
RF II
RF I
114S
(f)
cts/min x 10⁻³ —o—
PFU/ml x 10⁻⁹ --x--
fraction no.
(c) gene B
RF-II
RF-I
114S
(d)
cts / min x 10⁻³ —o—
fraction no.
(g) gene D
RF II
RF I
114S
(h)
cts/min x 10⁻³
fraction no.

FIG. 1. *a–h*.

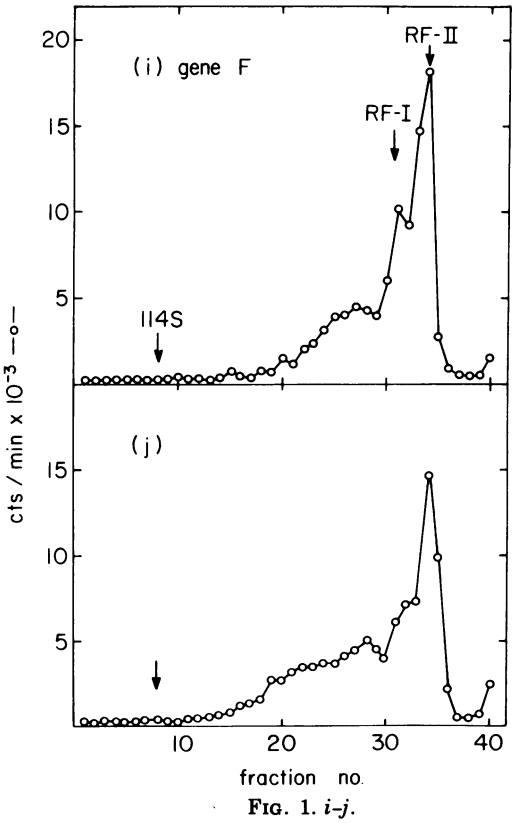

FIG. 1. *i-j.*

FIG. 1. *Sedimentation profiles of [³H]DNA in extracts of cells infected with mutants. Mitomycin C-treated E. coli HF4704 was infected with various mutants at 37°C at a multiplicity of infection of 7. [³H]thymidine (5 μCi/ml) was added to the culture at 11 min after infection and chased at 12 min after infection by the addition of unlabeled thymidine (200 μg/ml) and thymine (50 μg/ml). Samples were taken at 12.5 min (a, c, e, g, and i) and 16 min (b, d, f, h, and j) after infection. Extracts were prepared and centrifuged through 5 to 30% sucrose gradients in an SW41 rotor at 39,000 rpm for 150 min at 5°C as described in the text. (a and b) E mutant (N11)-, (c and d) B mutant (H210)-, (e and f) C mutant (oc6)-, (g and h) D mutant (H81)-, and (i and j) F mutant (H57)-infected cell extracts. (a, c, e, g, and i) 0.5-min chase; (b, d, f, h, and j) 4-min chase.*

the overall profile of the count distribution (Fig. 1d, h, and j). Cells infected with a gene G mutant gave a result similar to that with B, D, or F mutant-infected cells (data not shown).

Sedimentation analysis of DNAs. [³H]thymidine-labeled materials from various regions of the gradient shown in Fig. 1 were digested with Pronase in the presence of SDS, and the purified DNAs were analyzed by centrifugation in neutral sucrose gradients of low ionic strength (see Materials and Methods). [³H]DNA at fractions 34 and 32 of Fig. 1e sedimented at the positions of RFII and RFI, respectively (Fig. 2a and b). (Also, DNAs from the corresponding fractions of Fig. 1a, c, g, and i yielded RFII and RFI [data not shown].) [³H]DNA in the broader peak (fractions 22 to 28 of Fig. 1a, d, f, h, and j) was characterized by the presence of DNA sedimenting between marker RFI and RFII (Fig. 3a through e). These DNAs sedimented at the leading edge of RFI in a preformed CsCl sedimentation gradient (data not shown) as reported previously by Funk and Sinsheimer (12). They concluded that this DNA was an intermediate of viral DNA synthesis, i.e., an RFII molecule with a single-stranded tail (σ structure), because the σ structure isolated from cells infected with wild-type phage sedimented at the same position in the gradient.

In our gradient system, the σ structure sedimented between RFI and RFII as described previously (10). However, more detailed analysis of the DNA isolated from B, C, D, or F mutant-infected cells and sedimenting between RFI and RFII (hereafter called RFI*) revealed that RFI* has a different molecular configuration from the σ structure.

When RFI*, isolated from cells infected with B, C, D, or F mutants, was digested by S1 endonuclease (specific for single-stranded

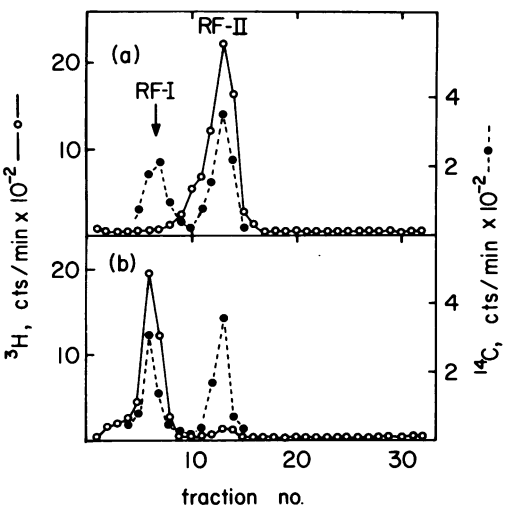

FIG. 2. *Neutral sucrose gradient analysis of [³H]DNA from material sedimenting near the top of gradient of Fig. 1e. Fractions 34 (a) and 32 (b) of Fig. 1e were treated with Pronase in the presence of SDS and centrifuged through a 5 to 20% neutral sucrose gradient at 49,000 rpm for 180 min at 20°C in an SW50.1 rotor as described in the text. A mixture of ¹⁴C-labeled RFI and RFII was added as a marker. Symbols: ○, ³H; ●, ¹⁴C.*

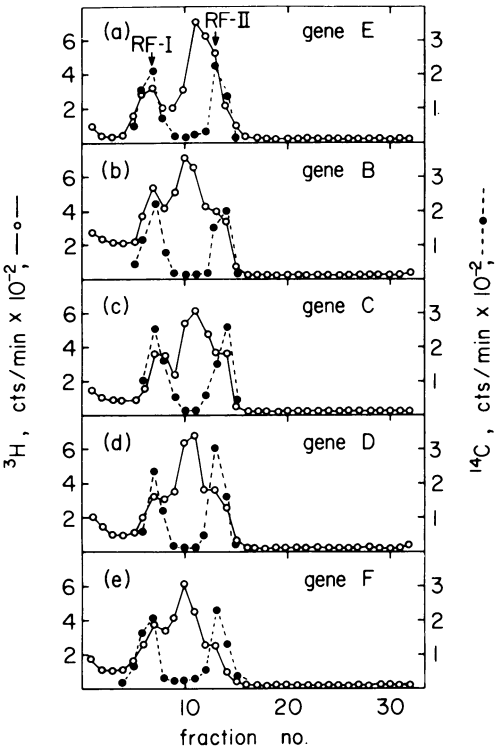

FIG. 3. *Neutral sucrose gradient analysis of [^{3}H]DNA from material sedimenting in the 40 to 60S region of Fig. 1. Fractions for no. 22 to 28 of Fig. 1 (a, d, f, h, and j) were collected separately, treated with Pronase in the presence of SDS, and centrifuged as described in the legend of Fig. 2. Symbols: ○, ^{3}H; ●, ^{14}C. (a) E mutant (0.5-min-chased sample from Fig. 1a); (b) B mutant (4-min-chased sample from Fig. 1d); (c) C mutant (4-min-chased sample from Fig. 1f); (d) D mutant (4-min-chased sample from Fig. 1h), and (e) F mutant (4-min-chased sample from Fig. 1j).*

DNA) and centrifuged in a neutral sucrose gradient, it was converted to RFII, but no digested materials were detected near the top of the gradient (Fig. 4b through e). Digestion of the single-stranded tail would be expected upon treatment of σ structure DNA with S1 nuclease. In fact, the σ structure isolated from the 50S complex in cells infected with E mutant phage did produce digested materials remaining near the top of the gradient in addition to the peak of RFII (Fig. 4a). It is to be noted that, under the experimental conditions used here, only a small portion of RFI added as a marker prior to digestion was converted to RFII. It has been reported that S1 endonuclease, although specific for single-stranded DNA, can attack supercoiled circular double-stranded DNA at an unpaired region of the DNA, resulting in conversion of RFI to RFII (3, 16). The conditions employed in Fig. 4 are milder than the conditions that allowed the conversion of RFI to RFII (F. K. Fujimura, personal communication). Since RFI* isolated from cells infected with B, C, D, F, or G mutants showed the same properties in this and subsequent experiments, we will describe experiments with RFI* from C mutant-infected cells in the following section.

(i) RFI*, which had been isolated from fractions 10 to 12 shown in Fig. 3c (Fig. 5a), cosedimented primarily with denatured RFI in an alkaline sucrose gradient (Fig. 5b), whereas the σ structure produced heterogenous single-stranded DNA ranging from one genome to two genomes in length (10, 11). (ii) After denaturation and renaturation in formamide, RFI* was

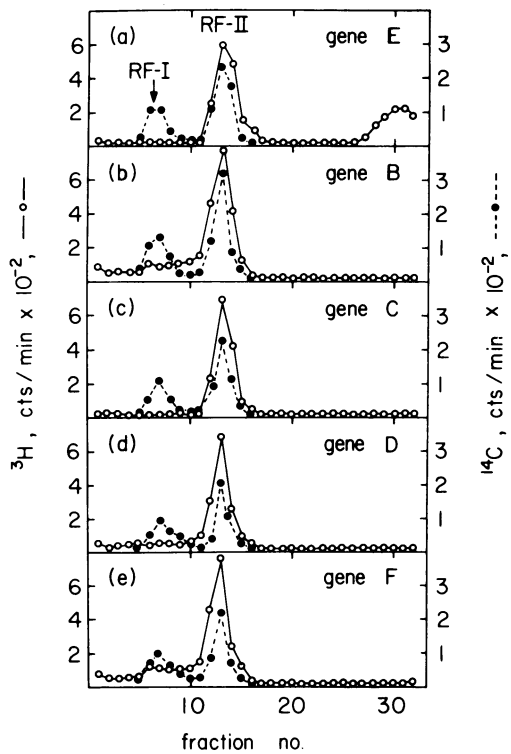

FIG. 4. *Neutral sucrose sedimentation of σ structure and RFI* structure after S1 endonuclease treatment. DNAs sedimenting between RFI and RFII of Fig. 3 were pooled separately, treated with S1 endonuclease, and centrifuged through a sucrose gradient as described in the legend of Fig. 2. ^{14}C-labeled RFI and RFII were added prior to S1 nuclease digestion. Sucrose gradients were fractionated directly into scintillation vials. Symbols: ○, ^{3}H; ●, ^{14}C. (a) E mutant (fractions 11 and 12 of Fig. 3a); (b) B mutant (fractions 10 and 11 of Fig. 3b); (c) C mutant (fractions 10 through 12 of Fig. 3c); (d) D mutant (fractions 10 and 11 of Fig. 3d), and (e) F mutant (fractions 9 through 11 of Fig. 3e).*

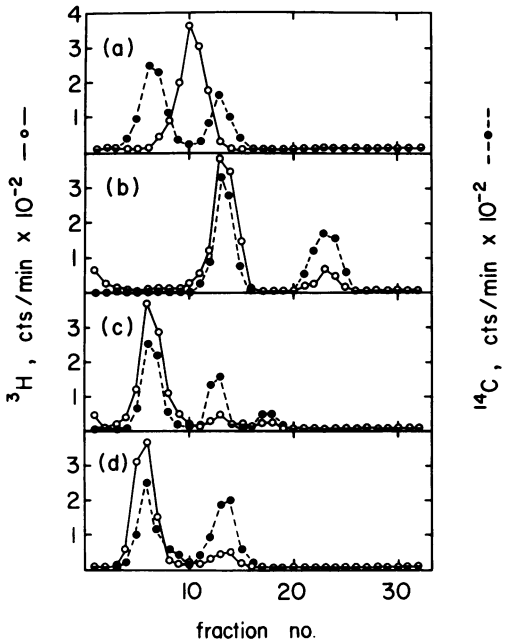

FIG. 5. *Characterization of RFI* from C mutant-infected cells. (a) [³H]DNA sedimenting between RFI and RFII in Fig. 3c (fractions 10 through 12) were pooled, dialyzed against EDTA buffer, and sedimented through a neutral sucrose gradient (5 to 30% in EDTA buffer) at 47,000 rpm for 180 min at 20°C. RFI* shown in (a) was sedimented through an alkaline sucrose gradient at 49,000 rpm for 90 min at 20°C (b). (c) Sedimented through a neutral sucrose gradient at 49,000 rpm for 180 min at 20°C after alkaline-denatured samples were made to 50% formamide (pH 7.5), incubated at 30°C overnight, and dialyzed against EDTA buffer. (d) Sedimented through a neutral sucrose gradient at 49,000 rpm for 180 min at 20°C after samples were treated with RNase A (50 μg/ml) for 1 h at 5°C in 0.03 M NaCl-0.015 M sodium citrate buffer. ¹⁴C-labeled RFI and RFII markers were added to the reaction mixture after the treatments (b through d). Symbols: ○, ³H; ●, ¹⁴C.*

converted to RFI (Fig. 5c). This result would not be expected from a similar treatment of the σ structure. (iii) RFI* was converted to RFI after incubation with RNase A at low ionic strength (Fig. 5d). However, extensive treatment of RFI* with Pronase in the presence of SDS and subsequent phenol treatment did not change its sedimentation property.

These results indicate that RFI* is a novel species of RFI complexed with RNA. In the experiments recorded in Fig. 5, a small amount of RFII was observed after treatment of RFI* with alkali, formamide, or RNase. The origin of the RFII is not clear at the present time.

Sedimentation profile of extracts from cells infected with mutant phages and labeled with amino acid. Cells infected with B, C, D, or F mutants were labeled with [³H]lysine between 10 and 20 min after infection and were chased for 4 min by the addition of an excess amount of unlabeled lysine. Extracts of the infected cells were prepared and analyzed (Fig. 6) by sucrose density gradient centrifugation as described in Materials and Methods. There was no radioactive peak in extracts prepared from cells infected with B, D, or F mutants except near the top of the gradients (Fig. 6a, c, or d). In C mutant-infected cells (Fig. 6b), two peaks (114S and 108S) were observed in addition to the top fraction. The 114S peak contained infectious phage particles. The 108S peak remained after DNase treatment of the extract and subsequent centrifugation. When the infected culture was labeled with [³H]thymidine, no peaks sedimenting at 108S were found (Fig. 1e), indicating that the 108S peak did not contain DNA. Protein species in the 108S peak were determined by SDS-polyacrylamide gel electrophoresis (Fig. 7). The relative amounts of F, G, and H proteins in the 108S peak are very similar to that of mature phage, indicating that the 108S peak may represent a structure similar to the capsid of mature phage. Furthermore, the 108S peak contained a large amount of D protein but lacked X protein, which exists in mature phage.

DISCUSSION

Nature of RFI*. Results of the pulse-chase experiments with [³H]thymidine in cells infected with a gene C mutant of φX174 (Fig. 1e and f) showed that pulse-labeled RFII was chased mainly into material sedimenting between 40S and 60S and that this material was characterized by the presence of RFI* (Fig. 3 and 4). RFI* is assumed to be an RFI molecule whose supercoiling has been relaxed by complexing (presumably by base pairing) with RNA. Such an assumption would explain the conversion of RFI* to RFI by denaturation with alkali, by denaturation and renaturation in formamide, and by RNase treatment at low ionic strength (Fig. 5). Also, RFI* might be expected to be more easily converted by S1 endonuclease to RFII than is RFI because of the unpaired single-stranded DNA in the region where the RNA is complexed (Fig. 4). Since it has been shown by both in vivo and in vitro experiments (21, 31) that RFI is more efficiently transcribed than is RFII, and since it has been known for some time that nascent mRNA is temporarily complexed with the template RF DNA (17–19), the RNA moiety attached to RFI* could be a por-

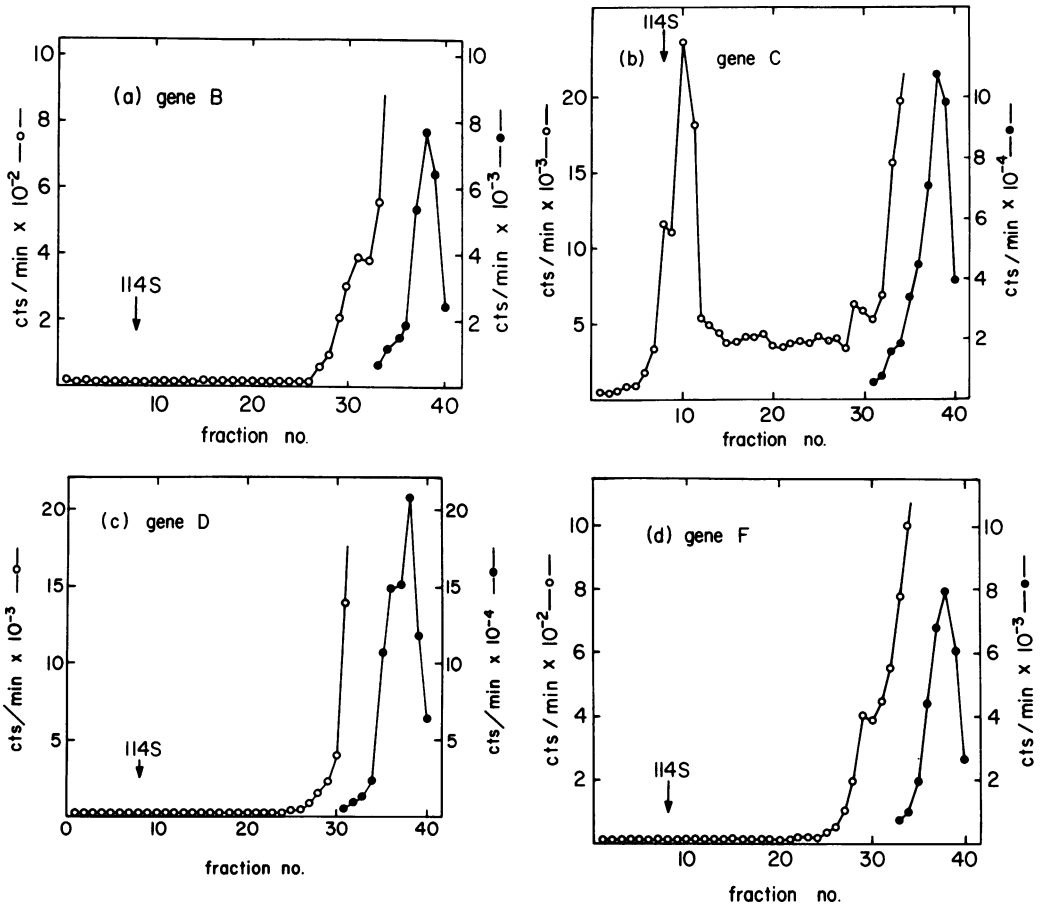

FIG. 6. *Sedimentation profile of extracts prepared from cells infected with mutants and labeled with [^{3}H]lysine. Mitomycin C-treated E. coli HF4704 was infected with mutants at 37°C at a multiplicity of infection of 7. [^{3}H]lysine (10 μCi/ml, 1 μg/ml) was added to the culture at 10 min after infection and chased at 20 min after infection by the addition of unlabeled lysine (200 μg/ml). Samples were taken at 24 min after infection, and cell extracts were prepared as described in Materials and Methods and centrifuged as described in the legend of Fig. 1. (a) B mutant-, (b) C mutant-, (c) D mutant-, and (d) F mutant-infected cell extract.*

tion of the newly synthesized mRNA transcribed by RNA polymerase after conversion of RFII to RFI. RFI DNA complexed with nascent mRNA through base pairing was originally isolated from wild-type-phage-infected cells. This DNA molecule sedimented differently from RFI or RFII and, after alkali treatment or RNase treatment at low ionic strength, the RNA moiety was digested, and the remaining DNA sedimented at the position of RFI. It thus has properties very similar to those of the RFI* described in this paper. RFI* has been isolated not only from cells infected with a gene C mutant, but also from cells infected with B, D, F, or G mutants. These facts indicate that RFI* is involved in a common process(es) existing in cells infected with these mutants. Whether RFI* does consist of RFI complexed with nascent mRNA awaits further experimental evidence. It should be noted that the superhelical template DNAs complexed with nascent RNA during transcription processes of simian virus 40 DNA in vivo (15) and in vitro (4, 25), or bacteriophage PM2 DNA in vitro (32), have been isolated. These complexes have properties very similar to those of RFI* and those of RF DNA and RNA complex reported previously (17–19). Richardson found that treatment of DNA-RNA polymerase-nascent RNA complexes with agents that denature or dissociate the RNA polymerase enhanced length of RNA complexing with the template DNA (32).

Although RFI* was observed in cells infected with B, D, or F mutants, RFII remained the

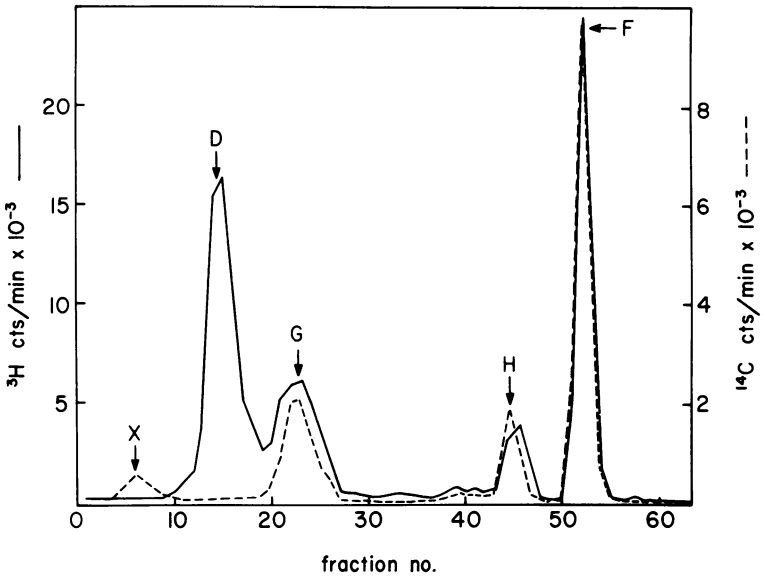

FIG. 7. *Polypeptide composition of 108S material in cells infected with C mutant. The 108S peak in Fig. 6b (fractions 10 and 11) was pooled and analyzed by SDS-polyacrylamide gel electrophoresis as described in the text. [¹⁴C]lysine-labeled phages were added as marker proteins.* ——, *³H;* ------, *¹⁴C.*

major species of DNA labeled after the 4-min chase (Fig. 1d, h, and i). It is known that RF replication continues in these cells (5, 23, 26, 27). Only phage-coded protein required for RF replication is gene A protein (9, 22, 23, 27), which catalyzes conversion RFI to RFII to allow further replication of RF (9, 22). RFI* existing in cells infected with B, D, or F mutants must have derived from RFI, a product of RF replication.

Since the conversion of RFII to RFI or to RFI* in cells infected with a C mutant must be preceded by strand closure of RFII, the chase of radioactivity from RFII to RFI* in cells infected with the gene C mutant suggests that the C gene product prevents RFI formation by not allowing the closure of RFII. Therefore, gene C product is apparently required at an early step in the synthesis of single-stranded viral DNA by maintaining the RF template in a functional state for viral DNA synthesis. In the absence of gene C product, closure of RFII to form RFI could occur.

The mechanism by which the C gene product maintains the open structure of RFII is not known. It is tempting to speculate that C protein might have DNA-binding properties and that the interaction of C protein at the nick (gap?, short tail?) in RFII prevents conversion to RFI. However, until some properties of gene C protein are determined, any number of models must be considered possible.

Production of small amounts of mature phage particles was observed in cells infected with the gene C mutant (Fig. 1f). These phages are not revertants because of their suppressibility. This low level of phage production may be due to leaky synthesis of gene C protein or to substitution of gene C function by host protein(s) as already speculated by Funk and Sinsheimer (12).

Nature of the 108S material. We previously found in cells infected with a wild-type phage that single-stranded DNA synthesis occurs in an intermediate complex that sediments with an *S* value of 50 in sucrose gradients (10). The 50S complex contains an RF molecule with an extended tail of single-stranded viral DNA and is a precursor of mature phage. When one round of viral DNA synthesis nears completion, cleavage of the growing viral strand occurs by the action of the gene A product(s) (11), resulting in the release of one molecule of RFII and a phage precursor. The RFII released after cleavage can be used repeatedly for the formation of new 50S complexes by associating with the appropriate protein structures. The present observation (Fig. 6) of the accumulation of 108S material in cells infected with a gene C mutant provides a possible candidate for a protein precursor of the 50S complex. The 108S material contained D, F, G, and H proteins but lacked X protein and DNA (Fig. 7). It was not detected in cells infected with B, D, or F mutants. It is possible that the 108S material might be an abortive aggregate that is formed when viral

DNA synthesis is extremely reduced. Alternatively, an interesting possibility is that the 108S material is a protein precursor of the 50S complex. Formation of the 108S material occurs independently of the C gene function. The C gene product maintains the availability of RFII as a template for single-stranded DNA synthesis. We could then envisage the aggregation of the 108S material with RFII to form the 50S complex before further elongation of viral strand DNA occurs. Whether or not the 108S material is a precursor or an abortive aggregate must await further experimental proof. (We have found accumulation of 108S material in cells infected with wild-type phage when single-stranded DNA synthesis was inhibited by thymidine starvation. Upon restoration of thymidine, the 108S material disappeared, and a concomitant increase in phage production was observed [H. Fujisawa and M. Hayashi, manuscript in preparation].)

Possible function of protein D. Siden and Hayashi (34) reported that five molecules of F protein and five molecules of G protein form a 12S particle when normal B gene function is provided. Since 12S particles were found in cells infected with D mutants, D protein may be required to promote and stabilize the aggregation of 12S particles (and H protein) to form a capsid structure (108S?). Contrary to this speculation, Weisbeek and Sinsheimer (39) proposed the possibility that D protein was involved in initiation of single-stranded DNA synthesis by protecting the nascent single-stranded DNA from being converted to double-stranded DNA. They assumed that D protein would have a function similar to the gene 5 protein of M13 (33). Gene 5 protein of M13 and fd (1, 30) can form an aggregate with single-stranded DNA. However, Farber (8) showed recently that D protein does not have a strong affinity for DNA.

As shown previously (10, 11), D proteins are associated with the 50S complex. Therefore, D protein should be released during morphogenesis since mature phages lack D protein. In fact, Weisbeek and Sinsheimer (39) found that 140S particles (our 132S particles) are mature phage associated with D protein. They showed that 140S particles can be converted to mature phage in vitro. Thus, D protein seems to be associated with presumed precursor particles: the 140S (132S) particles and the 50S complex. These facts suggest that D protein acts as a scaffolding protein for assembly and stabilization of capsid proteins during the assembly process of the phage. Supporting this speculation, 132S particles found in cells infected with an E mutant were not inactivated efficiently with antiserum against mature phage (114S) (Fig. 8). However, if 132S particles were first converted to 114S particles in vitro, the converted 114S particles were neutralized by the serum to the same extent as mature 114S particles. This observation indicates that in the 132S particles the completed virion is surrounded by D proteins in a scaffolding arrangement such that the D protein would prevent the phage antigens from reacting with the antiserum.

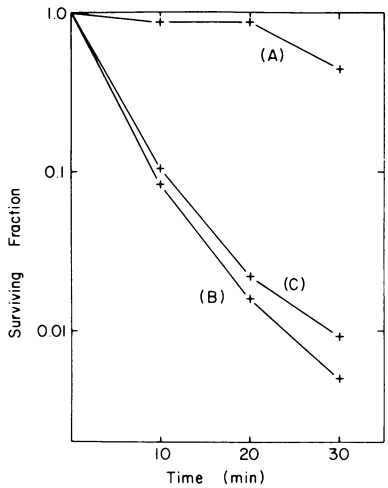

FIG. 8. *Antigenic property of 132S particles. Fractions 5 and 8 of Fig. 1a were used for 132S particles and 114S particles, respectively. $MgCl_2$ (final concentration, 20 mM) was added to a portion of the 132S fraction, and the mixture was kept at room temperature for 5 min to convert 132S to 114S. The 114S particles (B), 114S derived from 132S particles (C), and 132S particles (A) were neutralized with anti φX174 serum in EDTA buffer (K value, 0.4) and titrated on CR63.1 at different times after the addition of anti φX174 serum.*

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