Bacteriophage φ29 Proteins Required for In Vitro DNA-gp3 Packaging

MARY-ANN BJORNSTI, BERNARD E. REILLY, AND DWIGHT L. ANDERSON*

Departments of Microbiology and Dentistry, University of Minnesota, Minneapolis, Minnesota 55455

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In vitro assembly of bacteriophage φ29 in crude extracts involves efficient packaging of a DNA-protein complex (DNA-gp3) into a prohead with the aid of the gene 16 product (gp16) and subsequent assembly of neck and tail proteins (Bjornsti et al., J. Virol. 41:508–517, 1982; Bjornsti et al., J. Virol. 45:383–396, 1983; Bjornsti et al., Proc. Natl. Acad. Sci. U.S.A. 78:5861–5865, 1981). To define the viral proteins required for the DNA-gp3 encapsidation phase, we purified biologically active proheads and DNA-gp3 and constructed a chimeric plasmid, pUM101, which contained and expressed gene 16 of φ29 and no other viral genes. The plasmid-specified gp16 was both necessary and sufficient to package 24% of the DNA-gp3 added to the purified proheads, and the DNA-filled heads so produced were efficiently complemented to infectious phage by the addition of neck and tail proteins. Purified proheads and DNA-gp3 gave linear dose-response curves with slopes of approximately 1; in contrast, a 4-fold dilution of gp16 resulted in a 1,000-fold reduction of φ29, suggesting a requirement for multiple copies of this protein.

Efficient in vitro assembly is essential in understanding the molecular events of viral nucleic acid encapsidation, a general phenomenon. Bacteriophage φ29 is a particularly attractive model for such studies because in vitro assembly rivals in vivo assembly in efficiency (7–9). Also, the fibered anisometric head of φ29 and the intricate neck-tail reflect a complexity that belies a virion composed of only seven proteins (1, 5, 25, 31). Eighteen cistrons have been mapped that include several morphogenetic functions, and 25 virus-specific proteins, reflecting more than 90% of the coding capacity of the genome, have been identified (for reviews, see references 5 and 17).

In vivo and in vitro φ29 assembly a DNA-protein complex (DNA-gp3) is packaged with the aid of gp16 into a preformed prohead composed of the major head protein (gp8), the core-scaffolding protein (gp7), the neck-upper collar protein (gp10), and the dispensable head fiber protein (gp8.5). The neck lower collar (gp11), tail (gp9), and neck appendages (gp12*) are subsequently assembled onto the DNA-filled head in a linear sequence (7, 8, 11, 22, 27, 28, 38).

In vitro φ29 DNA-gp3 encapsidation and assembly results in an average yield of 180 phage per prohead donor cell in complementation extracts (9 × 10^11 phage per ml) (7). Functional DNA-bound gp3 (34) is required for effective in vivo and in vitro phage assembly, and proteinase K-treated DNA-gp3 is not repackaged in vitro (8, 9). The presence of discrete lengths and sequences of DNA in particles derived from packaging intermediates indicates a quantized packaging of DNA-gp3 which is oriented from left to right with respect to the genetic and restriction maps (9). Left-end restriction enzyme-generated fragments of DNA-gp3 are selectively packaged from a mixture of fragments, and the particles produced lack the core-scaffolding protein gp7 (9).

Presently we have defined all of the φ29 components required for the DNA-gp3 packaging phase of in vitro assembly. Proheads and DNA-gp3 have been purified with retention of high biological activity. Gene 16 of φ29 has been isolated and cloned in a chimeric plasmid, and gp16 is the only viral protein needed to efficiently and effectively encapsidate DNA-gp3. The proheads and DNA-gp3 gave first-order dose-response curves, but the gp16 dose response was of a higher order, indicating a requirement for multiple copies of this protein.

MATERIALS AND METHODS

Enzymes and isopes. The restriction endonucleases EcoRI and XbaI, bacterial alkaline phosphatase, phase λ DNA, and the plasmid pUB110 were from Bethesda Research Laboratories. T4 DNA ligase was from Promega Biotec. [3H]thymidine (TRK 637; 45 Ci/mmol) and [35S]methionine (SJ 204; 100 Ci/mmol) were from Amersham Corp. See reference 1 for other chemicals, enzymes, and isotopes.

Phage, bacteria, and plasmids. Phage φ29 (1, 31), reference mutants (23), the nonpermissive host Bacillus subtilis spoA12 (32), and the permissive host B. subtilis sup* (44) (24) were used. The recombinants sus7(614)-sus8(769)-sus14 (1241), sus4(369)-sus8(22), and sus7(614)-sus8(22)-sus16 (300)-sus14(1241) have been constructed for this work. The gene 4 product is required for late transcription, and the mutation sus14(1241) provides delayed lysis. As a source of gp16 we have constructed a chimeric plasmid pUM101 from the plasmid pUB110 of Staphylococcus aureus (13). φ29 DNA extracted from CsCl-purified phage (1) was digested with the restriction endonuclease EcoRI. The end fragments A and C were removed by adsorption to a glass filter (37), and the fragments B, E, and D were ligated as a mixture into EcoRI-cleaved, alkaline phosphatase-treated (39) pUB110. The DNA mixture was introduced into B. subtilis spoA12 by protoplast transformation (12), and clones resistant to neomycin (5 µg/ml) were then tested for gp16 function (32) and activity in vitro complementation. pUM101 was introduced into B. subtilis CU403 (thyA thyB metB divIVB1) (30) by protoplast fusion. The minicell-producing strain containing pUM101 was then tested as a source of gp16 in vitro complementation and gave a yield of 5.4 × 10^11 PFU/ml.

* Corresponding author.
TABLE 1. Purified proheads and plasmid-specified gp16 are effective in in vitro assembly

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<tr>
<th>Source</th>
<th>Assembly (PFU/ml × 10^11)</th>
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<tr>
<td>gp16</td>
<td>Proheads</td>
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<tr>
<td>7' 8&quot;14</td>
<td>16' 14&quot;</td>
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<tr>
<td>7' 8&quot;14</td>
<td>Prohead(^a)</td>
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<td>Plasmid</td>
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\(^a\) The complete reaction mixture (100 µl), prepared by combining equal volumes of extracts of cells infected with mutants 7' 8"14- (supplying gp16 and DNA-gp3) or 16' 14" (supplying proheads and DNA-gp3) was incubated at 23°C for 120 to 180 min (7).

\(^b\) Purified proheads (final concentration 3.7 × 10^12 or 7.3 × 10^12 ml) replaced the 16' 14" extract in the standard complementation.

An extract of cells carrying plasmid pUM101 (supplying gp16) replaced the 7' 8"14- extract in the standard complementation.

To define pUM101-directed protein synthesis, the microisolates were purified by the procedure of Reeve et al. (30) as modified by Shivakumar et al. (35). Minicells were labeled with [35S]methionine, except that a mixture of 17 amino acids (10 µg/ml of each) was added with 50 µCi of [35S]methionine per ml after preincubation for 20 min at 37°C. The proteins were identified after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2) and autoradiography.

Purification and quantification of proheads and radiolabeled DNA-gp3. To purify proheads, sus16(3000)-sus14 (1241)- or sus8(5900)-sus16(3000)-sus14(1241)-infected B. subtilis spoA12 was incubated in Difco antibiotic medium no. 3 (Penassay broth) containing 0.04 µCi of [35S]methionine per ml for 90 min at 37°C, concentrated 100-fold in Penassay broth by centrifugation, and treated with 1 mg of lysozyme per ml at 30°C until lysis. Cellular debris was removed by centrifugation at 12,000 × g for 10 min, and the supernatant was layered on 36-ml gradients of 5 to 20% (wt/vol) sucrose in TMS buffer (0.05 M Tris-hydrochloride [pH 7.8], 0.01 M MgCl₂, 0.1 M NaCl) and centrifuged in an SW27 rotor at 25,000 rpm for 2 h and 15 min at 23°C. Gradient fractions containing proheads were pooled, and the sucrose was removed over a period of about 48 h at 4°C by successive dialysis against 5% (wt/vol) sucrose in TMS buffer, 2% (wt/vol) sucrose in TMS buffer, and TMS buffer. The proheads were then concentrated in TMS buffer in a Micro-ProDiCon negative pressure dialysis concentrator (Bio-Molecular Dynamics) and stored at 4°C.

Prohead counts were made as described previously (3). Dilutions of purified 16' 14" proheads and 0.088-µm polystyrene latex particles (Dow Chemical Co.) in water were mixed. Triton X-100 was added to 0.1%, and the mixture was sprayed onto Formvar films. The specimens were shadowed with platinum, and micrographs were made of droplet patterns. The prohead stock (see Fig. 1 and 4 and Table 1) contained 3.7 × 10^14 ± 1.0 × 10^14 particles per ml, determined by counting a total of 1,751 proheads and 1,297 reference latex particles in 31 micrographs.

4' 8" [3H]DNA-gp3 was prepared in the nonpermissive host in Penassay broth containing 2 µCi of [3H]thymidine per ml as described previously (8), except that the azopirimidine was omitted. After 80 min of incubation, the infected cells were concentrated 15-fold by centrifugation, lysed with 500 µg of lysozyme per ml in 0.01 M sodium citrate-0.08 M K₂HPO₄-0.04 M KH₂PO₄ (pH 7.4) at 37°C for 5 min and vigorously vortexed for 15 s in the presence of 0.1 M NaCl to shear the host DNA (21). The DNA was centrifuged in CsCl in the Sorvall TV-865 vertical rotor at 79,000 × g for 40 to 46 h, and the viral DNA (1.699 g/cm³) was dialyzed against 0.01 M TE buffer (1× TE buffer is 0.05 M Tris-hydrochloride [pH 7.8], 0.01 M EDTA) on 0.025-µm membrane filters (Millipore Corp.) for 30 min just before addition to the in vitro complementation extract.

To determine the genome equivalents of φ29 [3H]DNA-gp3 added in the experiments of Fig. 4 and 5, proteinase K-treated viral DNA and lambda DNA standards were separated in agarose gels and quantified (9). The concentration of φ29 DNA was determined by comparison to the λ DNA standards, and the specific activity of the DNA ([3H] cpm per µg) was calculated.

In vitro complementation. Extracts of virus-infected or plasmid-containing cells were prepared as described previously (9), but φ29 antiserum was used only with infected cells. Extracts and components were mixed on Parafilm, and complementation continued for 120 to 150 min at ambient temperature. Details are described in the figure legends.

RESULTS

Isolation of Components of the in Vitro DNA-gp3 packaging system. In vitro φ29 assembly in a reaction mixture containing extracts of cells infected with mutants 8' 10" 14- (supplying gp16 and DNA-gp3) or 16' 14" (supplying proheads and DNA-gp3) can produce about 50% as many PFU per milliliter as in vivo complementation by the same mutants (7). In the present study, the mutant 7' 8"14- replaced 8' 10" 14- because the polarity exhibited by 10" infections in nonper-
mississi6 cells results in the production of very small amounts of the neck lower collar protein gp11 (4). The 7.8 14 \times 16^{-14} complementation yield of 6.0 \times 10^{11} \pm 2.7 \times 10^{11} PFU/ml was indistinguishable from the 8^{-10} 14^{-14} \times 16^{-14} complementation yield (Table 1, data not shown).

At least 35\% of ^{35}S-labeled proheads in extracts can be converted to phage during in vitro complementation, but proheads isolated in sucrose gradients are 10-fold less active (7; data not shown). However, when sucrose was removed slowly by multistep dialysis and the proheads were concentrated by negative pressure dialysis (see above), the proheads effectively replaced the 16^{-14} extract in complementation (Table 1). The yield of 4.8 \times 10^{11} \pm 0.6 \times 10^{11} PFU/ml was indistinguishable from the yield obtained with 16^{-14} extracts estimated to contain about half as many proheads (Table 1). The purified proheads gave a linear dose response with a slope of 1.1 when complemented with a 7.8^-14 extract (Fig. 1). When 3.7 \times 10^{10} purified proheads per ml were added, the virus yield was 4.5 \times 10^{10}/ml, and with dilution 15.7 \pm 1.1\% of the proheads were assembled to phage.

To provide the DNA packaging protein gp16 we constructed a chimeric plasmid from the S. aureus plasmid pUB110 (13) and fragments of \phi 29 DNA-gp3 generated by digestion with the restriction endonuclease EcoRI (see above). Viral genes of the chimeric plasmid pUM101 were defined by in vivo complementation (32). Phage yields after 16^{-14} infection of the pUM101-containing nonpermissive host were 1,000-fold higher than in 16^{-14} infection of the nonpermissive host and 10^{-1} mutant infection of the pUM101 host (data not shown). Marker rescue data indicate that the mutation sus16(300) is to the right of EcoRI fragment B of \phi 29 DNA (20). Moreover, plasmid pUB110 containing \phi 29 EcoRI fragment B can complement 11^{-}, 12^{-}, 13^{-}, and 14^{-} mutants.

FIG. 2. Expression of pUM101 in B. subtilis minicells. Minicells and minicells carrying pUB110 or pUM101 were purified and labeled with [^{35}S]methionine, and the proteins were extracted for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2, 35). Lane f shows [^{14}C]proteins in \phi 29, and lanes d, e, and g show [^{35}S]proteins in \phi 29 mutant-infected or uninfected, UV-irradiated B. subtilis spoA12 to position viral proteins. Lanes: (a) minicells without plasmids, (b) minicells carrying pUB110, (c) minicells carrying pUM101, (d) sus16(300)-sus14(1241)-infected cells, (e) sus7(614)-sus8(22)-sus10(302)-sus11(685)-sus14(1241)-infected cells, (f) CsCl-purified \phi 29, (g) uninfected bacteria.

FIG. 3. gp16 dose response. Equal volumes of extracts of 16^{-14} -infected cells and serial twofold dilutions of extracts of B. subtilis spoA12 carrying pUM101 were incubated for 120 min and the PFU per milliliter were determined (7).
but not a 16" mutant (data not shown). pUM101 contains the EcoRI fragments D and E of φ29 DNA (data not shown). gp16 provided by the plasmid gave a yield of 4.1 × 10^{11} ± 2.1 × 10^{10} PFU/ml in vitro complementation (Table 1).

To establish that gp16 was the only viral protein contributed by plasmid pUM101, the plasmid was transferred to B. subtilis CU403, a source of minicells, by protoplast fusion (12, 30; see above). The 35S-labeled proteins produced by minicells and minicells containing either PUB110 or PUM101 were examined by sodium dodecyl sulfate-polyacylamide gel electrophoresis (Fig. 2). Four PUB110-specified proteins were detected (Fig. 2, lane b), with molecular weights estimated at 49,000 (49K), 34K, 33.5K, and 13.5K when compared to φ29 proteins (19). The 49K protein was not produced by minicells carrying pUM101, and a new protein of 57K appeared (Fig. 2, lane c). A second new protein was identified as gp16 by comparison with proteins produced in 16" and 7" 8"10-11-14" infections of the nonpermissive indicator (compare lane c with lanes d and e in Fig. 2). The split gp16 band has also been observed in gels of infected cell lysates. pUM101 did not produce gp15 or gp17 (products of flanking genes) or any other phage protein in minicells (Fig. 2, lane c).

The dose response of plasmid-specified gp16 in the in vitro complementation shown in Fig. 3 was quite different from either the prohead (Fig. 1) or the DNA-gp3 dose response (see Fig. 5). When the gp16 of 6.3 × 10^{10} cell equivalents per ml was diluted 4-fold, there was a 1,000-fold decrease in virus yield (about 6.8 × 10^{11} to 4.3 × 10^{10} PFU/ml). The dose response given by 8"14"-infected cells as a source of gp16 in in vitro complementation yielded similar results (data not shown).

**Refined in vitro DNA-gp3 packaging system.** When exogenous [3H]DNA-gp3 is incubated for 80 min in the in vitro complementation mixture, treated with DNase I, and then centrifuged in a sucrose gradient, most of the DNase I-protected, trichloroacetic acid-precipitable [3H]DNA-gp3 and assembled φ29 cosediment and center on fraction 4 of the gradient (7). Phage maturation is complete at 80 min. When exogenous [3H]DNA-gp3 encapsidation is interrupted by DNase I treatment at 10 min, more than 28% of the [3H]DNA-gp3 that is ultimately packaged is positioned in the gradient peak centering on fraction 6, although very few phage (4 × 10^{7}/ml) are assembled (9).

A refined in vitro DNA packaging system consisted of purified 16"-14" proheads, 4"-8" [3H]DNA-gp3 isolated from infected cells, and an extract of cells containing pUM101 as a source of gp16. In this system the packaging as DNase I-protected material (about 24% of the [3H]DNA-gp3 added in complementation) was maximal at 70 min (Fig. 4, inset), and at this time about 10% of the [3H]DNA-gp3 added was found in fractions 4 through 8 of the sucrose gradient, the position of DNA-filled heads.

When exogenous 4"-8" [3H]DNA-gp3 packaged in the refined system for 70 min was complemented for 60 min with an extract of 7"-8"16"-14"-infected cells as a source of necktail proteins to complete assembly, 14% of the [3H]DNA-gp3 added in complementation sedimented as filled heads or phage, and an additional 11% of the label sedimented to fractions 10 through 29 of the gradient (Fig. 4). Of the 4.1 × 10^{10} PFU/ml assembled, 85% or 3.5 × 10^{10} PFU/ml contained the 4"-8" [3H]DNA-gp3 (577 of 680 plaques examined by qualitative complementation); thus about 6% of the 5.8 × 10^{11} exogenous DNA-gp3 equivalents per ml added was packaged and assembled to phage.

When the 7"-8"16"-14"-infected cell extract was added to the refined system at zero time and the mixture was incubated for 130 min, 15% of the exogenous [3H]DNA-gp3 added in complementation sedimented in the sucrose gradient as filled heads or phage, and an additional 13% of the label sedimented to fractions 10 through 29 of the gradient. Of the 7.9 × 10^{10} PFU/ml assembled, 20% or 1.6 × 10^{10} PFU/ml contained the 4"-8" [3H]DNA-gp3 (133 of 680 plaques examined by complementation) (data not shown).

To study the DNA-gp3 dose response in the simplified system, purified 8.5"-16" fiberless proheads (biologically indistinguishable from fibered proheads) and serial twofold dilutions of 4"-8" [3H]DNA-gp3 were incubated for 70 min with an extract of cells carrying pUM101 as a source of gp16. Portions of the reaction mixture were sedimented in sucrose gradients to quantify [3H]DNA-gp3 packaging, and other portions were complemented with 7"-8"16"-14"-infected-
ed cell extracts to convert filled heads to phage. The dose response (Fig. 5) had a slope of one and demonstrated that a constant proportion of the exogenous \(^3\text{H}\)DNA-gp3 added in complementation appeared in the gradient position of DNA-filled heads or phage. About 6.7 ± 1.1% of the exogenous DNA-gp3 added in complementation was packaged and sedimented to fractions 1 through 9, and 24.3 ± 6.1% of the \(^3\text{H}\)DNA-gp3 added appeared in fractions 1 through 29. When DNA-gp3 packaged at 70 min was complemented with extracts of 7\(^{8}\) 16\(^{14}\)-infected cells, the average virus yield was 6.7 ± 4.7 × 10\(^{10}\) PFU/ml and the proportion of 4\(^{8}\)8\(^{-}\) phage in the lysate decreased as a function of 4\(^{8}\)8\(^{-}\) DNA-gp3 dilution (Fig. 5).

**DISCUSSION**

In vitro assembly of \(\phi 29\) in crude extracts is remarkably similar to in vivo assembly in terms of the relative proportions of precursor components assembled, the amount and types of aberrant structures present, and the overall efficiency of DNA-gp3 encapsidation and subsequent neck-tail assembly (7; see above).

When proheads were purified by sucrose gradient centrifugation, the only viral proteins detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were gp7, gp8, gp10, and gp8.5. Densitometer tracings of photographic negatives of Coomassie blue-stained gels showed that more than 96% of the material was prohead protein (data not shown). These purified 16\(^{14}\) proheads gave yields of 4.8 × 10\(^{11}\) PFU/ml in vitro complementation (Table 1), and the dose-response curve was linear with a slope of 1.1 (Fig. 1); 15.7 ± 1.1% of the proheads could be assembled to phage in a complementation with a yield of 4.5 × 10\(^{11}\) PFU/ml. Considering that the typical efficiency of utilization of proheads both in vivo and during in vitro complementation is from 30 to 50% (7, 28), half of the proheads used in this dose response might have been competent for the DNA packaging phase of assembly.

The pUM101-specified gp16 could replace the extract of 7\(^{8}\) 16\(^{14}\)-infected cells in vitro complementation with a yield of about 4.1 × 10\(^{11}\) PFU/ml (Table 1). When the concentration dependence of gp16 was examined with plasmid-specified gp16 or infected cell extracts, the results suggested that multiple copies of gp16 may be required for packaging (Fig. 3; data not shown). A 4-fold dilution of the plasmid-specified gp16 extract resulted in a 1,000-fold decrease in viral yield. The decrease in viral yield was not due to dilution of essential host proteins, because the gp16 was assayed in a constant amount of an extract of 16\(^{14}\)-infected cells (Fig. 3). gp16 is probably not inactivated by dilution. An extract with plasmid-specified gp16 was diluted 32-fold, and the yield was reduced to 1.3 × 10\(^{8}\) PFU/ml; the diluted extract was concentrated by centrifugation in a Centricon-30 microconcentrator with a 30,000 \(M\) cutoff membrane. Thirty-one percent of the gp16 biological activity (1.3 × 10\(^{11}\) PFU/ml) was recovered when the diluted extract was concentrated 32-fold (data not shown). We have not derived the \(n\) number for gp16 from the concentration-dependence curve because our assay is relatively insensitive and we are purifying gp16. Without implying common mechanisms or functions, multiple copies of gp16 of \(\phi 29\) and the proteins gp3 (phage \(\lambda\)), gp2 (phage \(P2\)), and gpM (phage \(P2\)) are required for each productive packaging event (6, 10, 29).

Although about 30% of the exogenous \(^3\text{H}\)DNA-gp3 that is heat extracted from phage can be repackaged in the crude complementation system and can compete with endogenous DNA-gp3 with equal facility (7), the typical proportion of exogenous DNA-gp3 repackaged is only 2.8 ± 1.6% (\(n = \frac{9}{9}\)). Thus the conformation of the DNA-protein complex of the heat-extracted DNA might determine the packaging efficiency of a given preparation, and maintenance of the native DNA-gp3 conformation is important. Proteinase K digestion of the DNA-gp3 effectively blocks recombination (9). Because of the low efficiency of exogenous DNA-gp3 recombination, problems of DNA-capsid aggregation, and cognizance of the role of functional gp3, the DNA-gp3 used in the present experiments was prepared from infected cells lacking capsid proteins.

Purified proheads and \(^3\text{H}\)DNA-gp3 and a cell extract containing plasmid-specified gp16 comprise the refined in vitro packaging system. The efficiency of this system can be evaluated by sucrose gradient centrifugation of DNase I-protected \(^3\text{H}\)DNA-gp3 or by subsequent complementation of filled heads to \(\phi 29\). For example, 14% of the \(^3\text{H}\)DNA-gp3 added in complementation in the experiment of Fig. 4 sedimented as filled heads. This is an underestimate of DNA encapsidation because nascent DNA-filled heads are unstable in the absence of neck-tail assembly (Fig. 4, inset), and about one-half of the \(^3\text{H}\)DNA-gp3 in filled heads is invari-
ably lost to fractions 10 through 29 of the sucrose gradient during centrifugation (Fig. 4) (7–9), becoming DNase I sensitive (data not shown). In the experiment of Fig. 4, 25% of the [3H]DNA-gp3 added in complementation was DNase I protected before centrifugation. Packaging efficiency in the refined system was slightly less than that of the crude complementation system (7–9), in which an average of 20% (range, 12 to 29%; n = 6) of the DNase I-protected [3H]DNA-gp3 sedimented to the position of DNA-filled heads in sucrose gradients. However, only about 50% of the intracellular DNA-gp3 is packaged during infection (7, 8), and in vitro complementation in the crude system is about one-half as efficient as in vivo complementation with identical mutants (7).

When DNA-gp3 encapsidation and neck-tail complementation occurred simultaneously in the refined system, the yield was 7.9 × 10^10 PFU/ml. Assuming 500 copies of endogenous DNA-gp3 per cell equivalent in the 7–8 × 14–16 complementation extract, the ratio of exogenous to endogenous DNA-gp3 in the extract was 0.18, and the ratio of these DNAs in the progeny phage was 0.2 (data not shown). Thus the 4–8 DNA-gp3 molecules that could be packaged competed effectively with endogenous DNA, as exogenous DNA also does in crude complementation (7, 9). In addition, a constant proportion of 4–8 exogenous DNA-gp3 was packaged on dilution, and the 4–8 phase in the yield accurately reflected this dilution (Fig. 5). Even though an average of 24% of the exogenous [3H]DNA-gp3 added was DNase I protected in the dose-response experiment (Fig. 5), we have not defined the proportion of the DNA-gp3 that can participate in packaging because of the instability of filled heads (Fig. 4, inset).

Other factors peculiar to this refined system may also affect DNA packaging or neck-tail assembly (or both). Empty heads accumulate in 13–16 mutant-infected cells (18, 22), and gp13 may have a morphogenetic role in gp9 function during neck-tail assembly (16); neither gp13 nor gp9 is present during refined DNA-gp3 packaging. In addition, analogs of ATP can block virus assembly in vitro (7), and the plasmid pUB110 (and possibly pUM101) produces a nucleotidyltransferase (probably the 34K protein of Fig. 2 [33, 35]) that could act as an ATPase and affect DNA-gp3 packaging or neck-tail assembly in these experiments.

A comparison of the φ29 and λ in vitro DNA packaging reactions illustrates the novelty and utility of the φ29 system. Approximately 0.05 to 0.5% of λ DNA added can be assembled to phase in refined and unfracti- onated extracts (14, 36), whereas 6 to 12% of the φ29 DNA-gp3 added in the present experiments was packaged and converted to phase. Purified phage T3 proheads and gp19 possess biological activity comparable to that of φ29 proheads and gp16 when assayed in unfracti- onated extracts (15, 36), but these T3 components apparently have not yet been combined productively in a refined system. Thus the φ29 system is presently most amenable to biochemical and biophysical characterization because the high efficiency permits the isolation of packaging intermediates (9). The refined φ29 system is being used to study the functions of gp16 and ATP, the native or active form of gp3 of DNA-gp3, the maturation and competence of proheads, and the encapsidation of DNA fragments and hybrids.

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LITERATURE CITED


