A New Procedure for the Purification of the Bacteriophage λ Terminase Enzyme and Its Subunits

PROPERTIES OF GENE PRODUCT A, THE LARGE SUBUNIT*

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New methods for the purification of highly active bacteriophage λ terminase holoenzyme, and its individual subunits, gene products (gp) A and gpNu1, have been developed. These methods are rapid, simple, reproducible, and give high yields of unaggregated protein from small volumes of culture. The procedures involve fractionation of extracts of *Escherichia coli* strains harboring plasmids engineered to overproduce the respective proteins. All purified proteins exist as monomers or dimers at moderate concentrations. At concentrations where holoenzyme efficiently promotes *in vitro* cosN-cleavage and λ DNA packaging, gpA displays neither of these activities unless supplemented with gpNu1 and the E. coli protein integration host factor. At high protein concentrations, however, gpA can promote cos cleavage by itself. Although gpNu1 itself cannot promote either cosN-cleavage or DNA packaging, it does modulate these activities of gpA. GpA is a DNA-stimulated ATPase whose catalytic parameters closely resemble those of the holoenzyme. Like the holoenzyme, gpA displays a DNA helicase activity which is able to melt the annealed cosN overhangs. Certain preparations of gpA appear to undergo a time-dependent amino-terminal clipping at discrete sites even in the presence of as many as four protease inhibitors and at low temperature.

The bacteriophage λ terminase is a multifunctional enzyme responsible for the maturation and packaging of λ DNA (for reviews, see Feiss (1986), Becker and Murialdo (1990), and Murialdo (1991)). Maturation *in vivo* of the concatemers of replicating λ chromosomes occurs by terminase cleavage at cos sites (where cos represents cohesive end site, composed of two portions: cosB and cosN), introducing two staggered nicks in cosN, regenerating the 12 base single-stranded ends characteristic of the mature molecule. During packaging, terminase specifically selects and binds empty λ proheads and processively packages the λ chromosome.

The purified enzyme (Gold and Becker, 1983) is composed of two subunits, the products of genes *Nu1* (gpNu1, *M* = 20,444), and A (gpA, *M* = 72,280). *In vitro*, as well as promoting the above mentioned activities, terminase is an ATPase (Gold and Becker, 1983), a DNA helicase (Higgins *et al.*, 1988), and is also involved in the late stages of packaging where other phage proteins play a role (Becker *et al.*, 1977). We have attempted to assign these various functions to domains in the enzyme molecule. Current ideas about their location arise from studies on hybrid phages, comparison of DNA and protein sequences, and characterization of spontaneous and induced mutants. The Nu1 subunit is thought to have a helix turn helix DNA-binding domain at its amino terminus (Feiss, 1986; Kypr and Mrazek, 1986), and indeed, the protein in *vitro* has been demonstrated to have specific DNA binding properties, as demonstrated by filter binding (Parris *et al.*, 1988), gel mobility shift (Yang, 1993), and footprinting experiments (Shinder and Gold, 1988).

GpNu1 specifically bound and protected from DNase I three similar sequences (R1, R2, and R3) in cosB, the terminase binding site of cos. The Nu1 sequence also contains canonical ATP binding and hydrolysis domains, and the protein exhibits some ATPase activity (Becker and Gold, 1988; Parris *et al.*, 1988). Its carboxyl-terminal domain has been shown to specify subunit assembly with gpA (Feiss, 1986). However, by itself, gpNu1 has neither packaging nor cleavage activity (Parris *et al.*, 1988).

In the A subunit, the amino-terminal 48 amino acids are important for the specificity of assembly with gpNu1 (Feiss, 1986); other sites include a putative ATP-binding domain (Guo *et al.*, 1987; Becker and Gold, 1988), a metal ion-binding site (Berg, 1986), and a prohead capture domain in the last 32 amino acids of the carboxyl terminus (Feiss, 1986; Wu *et al.*, 1988). It has been suggested that the endonucleolytic (DNA nicking) center resides in gpA (cited in Higgins *et al.* (1988)). This concept was reinforced by the discovery of mutations in the A gene which abolished terminase DNA cleavage but did not significantly alter packaging activity (Davidson *et al.*, 1991). One of these mutations was localized to a putative "leucine zipper" (Vinson *et al.*, 1989) DNA binding motif and another to a motif found in several DNA polymerases and other phage terminase systems (Davidson and Gold, 1992). These mutations flank the presumptive ATP reactive domain. Similar putative activity domains have also been seen in the closely related phage 21 (Davidson and Gold, 1992; Smith and Feiss, 1993).

Our previous attempts to characterize terminase, especially its physical properties, were hampered by the wild type enzyme's poor expression in *Escherichia coli* (Gold *et al.*, 1981). The original purification scheme adopted (Gold and Becker, 1983) yielded only about 0.4 mg of holoenzyme from 4600 g of frozen cell pellet and only after several weeks and many fractionation steps. The cloning and construction of overproducing plasmids of the *Nu1* and *A* genes separately and together (Keeler, 1987; Chow *et al.*, 1987; Murialdo *et al.*, 1987) was an extremely helpful development in this respect. However, gpNu1 prepared from overproduction was highly aggregated, and although completely soluble in solutions of high ionic strength, molecular size determinations indicated a molecular mass greater than 106 daltons for a 21-kDa protein (Parris et
Bacteriophage \(\lambda\) Terminase Subunits

Similar difficulties were also encountered with terminase. In the availability of a large number of different kinds of mutants distributed throughout both subunits (Davidson et al., 1991) and the fact that some of terminase's many activities could only be assessed in vitro using holoenzyme in some instances and the individual subunits in others, and that certain activities could not be reliably assayed in crude extracts, we attempted to develop new protocols for more easily and quickly obtaining reasonable quantities of pure proteins. In this report we describe these procedures which can reproducibly give high yields of active, nearly homogeneous, unaggregated holoenzyme and subunits. Also, the properties of the isolated gpA subunit are here presented for the first time. Under conditions where terminase can actively promote those activities, gpA has neither in vitro cos-cleavage nor \(\lambda\) DNA packaging activity unless complemented with gpNul; however, gpA itself is an active ATPase and DNA helicase whose properties are quite similar to those of the holoenzyme. Under certain other conditions, however, we show that gpA is in its own right a specific endonuclease whose properties can account for much of the cos-cleavage activity of terminase. Details of gpA's ATPase and endonuclease activities are presented in the accompanying papers (Rubinchik et al., 1994a, 1994b).

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains**—The bacterial strains and the plasmids constructed to overexpress holoenzyme, pCM101; and the individual subunits gpA, pCM2 and CK1131, and gpNul, CR1213, pCM24, and pCM35 (Chow et al., 1987; Keeler, 1987; Murielado et al., 1987), have been described previously. All of these plasmids overproduce the terminalase. DNA material extracts (crude) are named after the plasmid harbored in that strain.

**Overproducing Strains and Preparation of Extracts**—Bacteria for preparations of terminase, gpA, and gpNul extracts were grown, induced, and harvested as described in Chow et al. (1987). A HpaII subfragment of the pWP14 gene, in place of their own in order to overcome poor transformation efficiency, was added last in various dilutions. The reaction volumes were 15 \(\mu\)l, and the reaction buffer contained 20 \(\mu\)l Tris-HCl, pH 8.0, 5 \(\mu\)l MgCl\(_2\), and 2 \(\mu\)l ATP. The reactions were incubated at 37 °C for 20 min, and stopped by the addition of 3 \(\mu\)l of 60% glycerol, 250 \(\mu\)l EDTA, 5% SDS, and 0.01% bromphenol blue. For this assay supercoiled substrate DNAs (pWP14, or pSR6) were first limited with an appropriate single-cutting restriction endonuclease. When a successful cos-cleavage has occurred distinctive DNA fragments are generated which can be separated by agarose gel electrophoresis and visualized with UV light after ethidium bromide staining. Quantitation of the activity was estimated by scanning photographic negatives of the gels.

**Footprinting Assays**—ATPase activity was determined spectrophotometrically (source as described previously (Parris et al., 1994)). Reaction volumes were 15 \(\mu\)l containing 6 \(\mu\)M Tris-HCl, 15 \(\mu\)M MgCl\(_2\), and 0.01% 2-mercaptoethanol, and 0.01% bromphenol blue. For this assay supercoiled substrate DNAs (pWP14, or pSR6) were first limited with an appropriate single-cutting restriction endonuclease. When a successful cos-cleavage has occurred distinctive DNA fragments are generated which can be separated by agarose gel electrophoresis and visualized with UV light after ethidium bromide staining. Quantitation of the activity was estimated by scanning photographic negatives of the gels.

**Assays of Cos-cleavage Activity**—Assays of cos-cleavage activity were performed essentially as described previously (Shinder and Gold, 1988). 32P-labeled triphosphates and products analyzed using polyethyleneimine-cellulose thin layer chromatography (TLC) as previously described (Gold and Becker, 1983). The TLC plates were then exposed to phosphor crystal screens compatible with the PhosphorImager system from Pharmacia LKB for 16 h. The plates were then scanned by the PhosphorImager and the images analyzed by the Molecular ImageQuant software (Johnston et al., 1990). Alternatively, a coupled spectrophotometric assay with lactate dehydrogenase was employed (Jenkins, 1991), when unlabeled ATP was used. Assays of Cos-cleavage Activity—Assays of cos-cleavage activity were performed essentially as described previously (Shinder and Gold, 1988). 32P-labeled triphosphates and products analyzed using polyethyleneimine-cellulose thin layer chromatography (TLC) as previously described (Gold and Becker, 1983). The TLC plates were then exposed to phosphor crystal screens compatible with the PhosphorImager system from Pharmacia LKB for 16 h. The plates were then scanned by the PhosphorImager and the images analyzed by the Molecular ImageQuant software (Johnston et al., 1990). Alternatively, a coupled spectrophotometric assay with lactate dehydrogenase was employed (Jenkins, 1991), when unlabeled ATP was used. Assays of Cos-cleavage Activity—Assays of cos-cleavage activity were performed essentially as described previously (Shinder and Gold, 1988). 32P-labeled triphosphates and products analyzed using polyethyleneimine-cellulose thin layer chromatography (TLC) as previously described (Gold and Becker, 1983). The TLC plates were then exposed to phosphor crystal screens compatible with the PhosphorImager system from Pharmacia LKB for 16 h. The plates were then scanned by the PhosphorImager and the images analyzed by the Molecular ImageQuant software (Johnston et al., 1990). Alternatively, a coupled spectrophotometric assay with lactate dehydrogenase was employed (Jenkins, 1991), when unlabeled ATP was used.

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**Packaging Assays**—In vitro packaging assays were performed essentially as described previously (Parris et al., 1988) with the following adaptations. Approximately 1 \(\mu\)g of mature \(\lambda\) DNA (4 \(\mu\)l) was added to 50 \(\mu\l of buffer A (20 \(\mu\)l Tris-HCl, pH 8.0, 10 \(\mu\)l MgCl\(_2\), 1 \(\mu\)l EDTA, 3 \(\mu\)l 2-mercaptoethanol) containing 6 \(\mu\)M Tris-HCI, 15 mM ATP, 18 mM MgCl\(_2\), 60 \(\mu\)M spermidine-HCl, 30 \(\mu\)l 2-mercaptoethanol, and 20 \(\mu\l of a crude extract of pCM230 (source of proheads, gpD, gpW, gpFII, proheads, tails, etc.) were added. In gpA complementation assays, 10 \(\mu\)l of an extract of pCM24 was added as a source of gpNu1. In gpNu1 complementation assays, 10 \(\mu\)l of an extract of pCM2 was added as a source of gpA. Terminase, or gpA or gpNu1, as indicated was added last in various dilutions. The reaction was allowed to proceed at room temperature for 1 h. Mature infective phage particles were titrated on Q5005, and the results expressed as pfu/ml reaction mixture or units of \(10^6\) pfu/ml.

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were at 15,000 rpm for 60 min (Sorval RC2B, SS34 rotor) unless otherwise indicated.

Buffers and Columns—Mono Q HR5/5, 1 ml; HR10/10, 5 ml; and Superox 6 columns were purchased from Pharmacia and maintained as suggested by the manufacturer. The Econo-Pac Heparin cartridge (5 ml) was from Bio-Rad. All columns were operated in a Pharmacia FPLC system. A 50-ml superloop was used to apply samples to the Mono Q column. Chromatography was routinely carried out with back pressures of 1.5–3.5 megascalps. The Superox 6 column was operated with back pressure of 0.8 megascalps. Column buffers, the superloop, the column, and column fractions were kept ice cold during the purification.

Protein Sequencing—Samples were prepared for protein sequencing in the following manner. 12.5% SDS-PAGE gels were prepared according to Laemmli (1970) and subjected to a prerun of 2 h in 1 × separating gel buffer. Protein samples were run in 1 × reservoir buffer. After the run was complete, the protein bands were electroblotted onto polyvinylidine difluoride by standard methods (Le Gendre and Matsudaira, 1989) and the protein bands excised from the membranes. Protein sequencing was performed by the Protein Analysis Service, Department of Biochemistry, University of Toronto, using an Applied Biosystems 470A Gas-phase Sequenator coupled to a Model 120A phenylthiohydantoin-derivative analyzer.

Other Materials and Methods—Triton X-100, myoglobin, and protease inhibitors were from Sigma. Bacterial whole cell lysate from E. coli was from Boehringer Mannheim. SDS-polyacrylamide gels were run on a 7.5 or 12.5% gel as published by Laemmli (1970) with a Pharmacia Phast system according to manufacturers instructions and stained with Coomassie Brilliant Blue R. Sucrose density gradient centrifugation was performed using 10–30% linear gradients containing 0.02 M Tris-HCl, pH 8.0, 0.5 mM EDTA, and 5 mM dithiothreitol, and protease inhibitors as described in the gpA protocol. Centrifugation was for 17 h at 40,000 rpm at 15 °C in polyallomer tubes of a Beckman SW60 rotor. Thirty seven fractions of 8 drops each were collected. GpA and terminase activities were determined by cos-cleavage assays and by protein determination. Other sedimentation velocity measurements were performed in a Beckman Model E ultracentrifuge at 64,000 rpm for 50 min by the Protein Analysis Service, Department of Biochemistry, University of Toronto. Protein determinations were done using a Bio-Rad protein assay kit. Conductivity measurements were performed on a Radiometer Conductivity Meter. X-ray film employed was X-Omat-AR from Kodak.

IHF was purified by a modification of the method of Nash et al. (1987). L-Lactate dehydrogenase was purified from a cloned Bacillus stearothermophilus gene as described previously (Kallwass et al., 1992).

RESULTS

Purification of Terminase—A crude extract prepared by sonic disruption (Gold and Becker, 1983) from 1 g wet weight cells, clarified by centrifugation at 7,000 rpm for 10 min, was slowly brought to 20% saturation with solid ammonium sulfate. The precipitate was collected by centrifugation and discarded. Additional ammonium sulfate was added slowly to the supernatant to bring it to 45% saturation, and left on ice for at least 1 h. The precipitate was collected by centrifugation and resuspended in a minimum volume of buffer A with no MgCl₂ (ASI fraction).

The ASI fraction of terminase was diluted with Mono Q start buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol) to bring it to a conductivity of less than 6 mmho. The diluted fraction was loaded on a Mono Q HR5/5 column in the above buffer, and then washed with 6 ml of start buffer containing 0.3 M NaCl. A typical load for the column was 10 mg of protein. The column was developed with a 25-ml gradient from 0.3 to 0.6 M NaCl. Terminase eluted as a peak at approximately 0.48 M NaCl. Some terminase eluted in the pass through fractions, but these fractions were found to have little or no activity in in vitro packaging assays, and were discarded. Fractions were stored at ~70 °C until use. Nucleic acids eluted in a fraction at greater than 0.7 M NaCl. A typical chromatogram can be seen in Fig. 2 and a summary of the purification appears in Table I; the recovery of activity is difficult to quantitate because packaging is inhibited in crude extracts. Approximately 2 mg of pure terminase were obtained from a mass of cell pellet derived from approximately 1 liter of bacterial culture.

Characterization of Terminase—The terminase Mono Q fraction was estimated to be more than 95% pure on the basis of SDS-PAGE and Coomassie Blue staining. The A₂₆₀/₂₈₀ ratio, an indicator of nucleic acid content (Layne, 1957), increased from 0.6 in crude extracts, to 1.6, and even as high as 2.0 in some preparations. When 30 µl of an active Mono Q fraction (A₂₆₀ = 0.8, possible nucleic acid content 1.2 µg) were electrophoresed on an agarose gel and stained with EtBr, no nucleic acid was visible. A band with as little as 10 ng would have been detected by this method. Large amounts of nucleic acid elute much later in the gradient (data not shown).

When terminase was subjected to gel filtration on a Superose 6 column, it eluted in a position indicative of a protein of M₀ = 130,000 (Fig. 3, top). Molecular weight determinations by sucrose density gradient centrifugation indicated a molecular mass of 122,000 daltons (Fig. 3, bottom). These results are in keeping with our previous estimates of the composition of holoenzyme as a hetero-oligomer containing 2 to 3 gpNu1 and one gpA subunit. A similar observation has been reached by Tomka
TABLE I
Summary of terminase purification

| Fraction    | Volume | Total protein | Total packaging activity | Specific activity A_{280nm} |
|-------------|--------|---------------|--------------------------|-----------------------------|
| Crude extract | 3.7 ml | 79.2 mg | 3.0 x 10^7 | 1.7 x 10^5 | 0.5 |
| AS I        | 1.0 ml | 12.0 mg | 1.5 x 10^7 | 8.3 x 10^6 | 1.1 |
| Mono Q fraction | 2.0 ml | 2.0 mg | 4.0 x 10^7 | 1.0 x 10^7 | 1.6-2.0 |

* 1 unit = 1 x 10^9 pfu.

FIG. 3. Determination of molecular weights of terminase, gpA, and gpNu1. Panel A, gel filtration. Mono Q fractions from terminase gpA and gpNu1 preparations were separately applied to a Superose 6 HR 10/30 column equilibrated and developed with 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 75 mM NaCl, and 5% glycerol. Closed circles represent the position of protein molecular mass standards, which were in order of decreasing molecular mass: thyroglobulin, 669 kDa; ferritin, 440 kDa; γ-globulin, 160 kDa; BSA, 68 kDa; ovalbumin, 43 kDa; chymotrypsinogen, 25 kDa; and RNase, 13.7 kDa. The labeled arrows show the position for terminase, gpNu1, and gpA, respectively, all prepared as described in the text. The unlabeled arrow indicates where previous preparations of terminase and gpNu1 from overproducers purified by other methods in our laboratory elute from the column. Panel B, sucrose density centrifugation. Mono Q fractions from terminase and gpA preparations were layered on separate sucrose density gradients and run as described under “Experimental Procedures.” Parallel gradients containing the molecular mass markers myoglobin (17.8 kDa), bacterial alkaline phosphatase (80 kDa), and aldolase (158 kDa) were centrifuged at the same time and their respective migration is indicated by the closed circles. Data points represent averages and standard deviations of two experiments.

and Catalano (1993) who purified terminase by a completely different procedure. In vitro packaging (Table I), cos-cleavage assays (Fig. 4), and ATPase assays of the pure preparations showed that the overproduced holoenzyme had activities similar to those previously published (Gold and Becker, 1983). The K_m for terminase ATPase was 64 μM and the turnover number, 75 min^-1. However, the ATPase activity of the overproduced enzyme differed in that its degree of DNA dependence or stimulation was considerably less than previously reported (Gold and Becker, 1983).

Purification of gpNu1—Sonicated cells were centrifuged at 7,000 rpm for 10 min and the pellet (containing more than 90% of the intracellular gpNu1 in an almost pure form), was collected. The insoluble pellet from 1 g of cells was washed with 4 ml of 0.2% Triton X-100 in buffer A (minus MgCl_2 and at pH 7.0). Insoluble material was collected by centrifugation at 7,000 rpm for 10 min. The remaining pellet was solubilized by occasional stirring in 15 ml of 0.5% Sarkosyl (minus MgCl_2 and at pH 7.0) for a minimum of 24 h at 4 °C. Any remaining insoluble material was removed by spinning at 15,000 rpm for 20 min.

The Sarkosyl was removed by gradual dialysis against successive changes of buffer A containing successively lower concentrations of Sarkosyl (0.3, 0.2, and 0.1%) for a 1.5-h duration in each buffer, ending in an overnight dialysis in buffer alone. The ratio of dialyzed material to buffer was kept at 1:100.

The dialyzed, solubilized pellet was loaded onto a Mono Q HR5/5 column in the same start buffer as for terminase, and then washed with 5 ml of 0.3 M NaCl. The column was developed with a 30-ml gradient from 0.3 to 0.9 M NaCl. GpNu1 eluted as a peak at approximately 0.42 M NaCl. Typically, 15 mg of protein were loaded on this column and a typical chromatogram can be seen in Fig. 5. Approximately 80% of the protein can be recovered from this column. A summary of this purification appears in Table I. A yield of 11 mg of protein was obtained from 1 liter of bacterial culture. The DNA packaging activity in the pellet before solubilization was severely inhibited.

Characterization—The purity of the gpNu1 fraction was found to be more than 95% on the basis of SDS-PAGE (Fig. 5). The A_{280nm} ratio increased from 0.5 to 1.68 over the purification. When 30 μl of the Mono Q II fraction (A_{280}, 1.4, possible nucleic acid content approximately 2 μg) were electrophoresed on an agarose gel, no nucleic acid was detectable by EtBr staining. On the Mono Q column, the nucleic acids elute much later in the gradient than gpNu1.

The sedimentation coefficient (s_{20w}) for gpNu1 as determined by analytical ultracentrifugation was 3 S, characteristic of proteins with an M_r of approximately 3.5-4 x 10^6. In addition, the elution behavior of gpNu1 on a Superose 6 column was indicative of a protein with a molecular weight in the order of 4.2 x 10^6 (Fig. 3). These results suggest that gpNu1 prepared by this procedure existed as a dimer.

In vitro packaging (Table II) and cos-cleavage complementation assays (data not shown) showed that these levels of activities were similar to those previously published for gpNu1 purified by a completely different procedure (Parris et al., 1988). ATPase activity was also similar to that of fractions previously isolated from the insoluble pellet, having a turnover number of
**FIG. 5. Purification of gpNu1.** Panel A, SDS-PAGE of gpNu1 fractions at various stages of purification. Migration is from left to right. Lane 1, sonicated cells, 1 μg of protein; lane 2, pellet suspension, 2.2 μg of protein; lane 3, solubilized pellet, 0.9 μg of protein; lane 4, Mono Q fraction, 0.8 μg of protein. Molecular mass markers are indicated by letters, and are; a, carbonic anhydrase, 30 kDa; b, trypsin inhibitor, 20.1 kDa; c, α-lactalbumin, 14.4 kDa. Panel B, Mono Q chromatography of gpNu1. The black arrow indicates the position of gpNu1 elution. A$_{280}$ is represented by the solid line and NaCl concentration by the dotted line.

**TABLE II** Summary of gpNu1 purification

| Fraction       | Volume | Total Protein | Total Packaging Activity | Specific Activity | A$_{280}$/NaCl |
|----------------|--------|---------------|--------------------------|-------------------|---------------|
| Pellet suspension | 4      | 45            | 2.8 x 10$^4$             | 66                | 0.5           |
| Solubilized pellet | 15     | 45            | 2.5 x 10$^6$             | 1.4 x 10$^5$      | 0.5           |
| Mono Q fraction  | 5      | 11            | 3.6 x 10$^6$             | 3.3 x 10$^5$      | 1.68          |

* Complementation for packaging with gpA.

$^b$ 1 unit = 1 x 10$^6$ pfu.

between 0.4 and 0.8 min$^{-1}$. A DNase I footprint (Fig. 6) showed that gpNu1 prepared by our new procedure bound to the R1, R2, and R3 sites in cosB as previously described (Shinder and Gold, 1988).

**Purification of gpA**—An AS1 fraction was prepared from 1 g of cell pellet exactly as described above for terminase. All buffers for the gpA purification contained the protease inhibitors leupeptin (1 μg/ml), pepstatin (0.7 μg/ml), benzamidine hydrochloride (0.5 mM), bestatin (50 μg/ml), and phenylmethylsulfonyl fluoride (0.2 mM). The AS1 fraction was dialyzed for 1 h against the Mono Q start buffer.

The AS1 fraction of gpA after dialysis was diluted approximately 5-fold with Mono Q start buffer to a conductivity of less than 1.8 mmo, and loaded on a Mono Q HR10/10 column and washed with 20 ml of buffer containing 0.15 M NaCl. The column was developed with a 30-ml gradient from 0.15 to 0.55 M NaCl. GpA eluted as a peak at approximately 0.42 M NaCl. As with terminase and gpNu1, nucleic acids eluted later at greater than 0.7 M NaCl. A typical load for this column was 15 mg of protein and a representative chromatogram can be seen in Fig. 7. Ice-cold glycerol to a final concentration of 20% was immediately added to the fractions as they eluted from the column. Fractions were stored at -70 °C until use. A summary of this purification appears in Table III. A yield of 2 mg of protein was obtained from 1 liter of cell culture.

**Characterization of gpA**—The purity of gpA on the basis of SDS-PAGE analysis was found to be more than 95% (Fig. 7).

The A$_{280}$/NaCl increased from 0.5 to 1.7 over the purification, and in a similar fashion to terminase and gpNu1, nucleic acid eluted from the Mono Q column at a much higher salt concentration than gpA.

When subjected to gel filtration on Superose 6, gpA eluted as a single peak indicative of a molecular weight of 80,000 (Fig. 3, top). Sucrose density centrifugation experiments indicated a molecular weight of 62,400 (Fig. 3, bottom).

Pure gpA was able to complement gpNu1 preparations (crude extracts) in both packaging (Table III) and cos-cleavage assays (Fig. 4). GpA at concentrations where the holoenzyme can efficiently catalyze cos-cleavage was unable to cleave on its own without the addition of a gpNu1 crude extract, or purified gpNu1 and IHF (Fig. 4, lanes 3-6). However, gpA was able to cleave DNA by itself at higher concentrations (Fig. 4, lanes 7-10), and also at sites other than in cosN (Rubinchik et al., 1994a).

To further investigate the properties of the gpA endonuclease, DNA fragments containing full cos, cosN only, and neither cosB nor cosN were used as substrates (Fig. 8). GpA (Fig. 8, lanes 2 and 6), mixes of gpA and gpNu1 (lanes 3 and 7), and the holoenzyme (lanes 4 and 8), cleaved the fragments containing cosN whether or not cosB was present. (GpNu1 on its own has already been shown not to possess any nucleolytic activity (Parris et al., 1988).) However no cleavage was observed in the absence of cosN (Fig. 8, lanes 10-12), implying that the endonuclease activity of gpA was cosN-specific. In a related experiment (Willis, 1992), the plasmid pBW3 (Feiss and Widner, 1982) was tested as a substrate for terminase. This plasmid contains λ sequences to the left of cosN, cosN, and the right side of cosB from the closely related phase 21 whose R1, R2, and R3 sites differ from those of λ in several positions (Miller and Feiss, 1985). Under identical experimental conditions and with equivalent amounts of terminase, almost as much cleavage was obtained with pBW3 as with any of the λ cosB substrates (data not shown). In an accompanying report (Rubinchik et al., 1994a) experimental evidence will be presented which shows that under certain conditions some properties of the gpA endo-
Bacteriophage λ Terminase Subunits

nuclease are essentially similar to those of the holoenzyme. Even though gpA is capable of cosN-cleavage, no specific binding to cosN or to any cosB sequence could be detected by DNase I footprinting under these conditions (Fig. 9). However, using this and other techniques, such as gel mobility shift assays (Yang, 1993), we have shown that gpA exhibits a strong but nonspecific capacity to bind to DNA (Fig. 9, lane 3). Holoenzyme, however (Fig. 10), did protect a large region, including specific residues in cosN, in a manner similar to that reported for terminase purified from induced lysogens (as cited in Feiss and Becker (1983)). Overproduced terminase has been reported to protect R4 unlike gpNu1 (Shinder and Gold, 1988). We have also observed this protection (Fig. 10) when MgCl₂ is present in the reaction mixture and the top strand is nicked at N2, as is normally the case in terminase assays. When MgCl₂ is substituted with Ca²⁺, terminase cannot cut efficiently (Rubinchik et al., 1994a). Under these conditions (Fig. 10), the protection of the region from R3 to the right half of cosN was stronger, but the footprint did not include R4. A low level of nicking at N2 was observed in this experiment since there was some MgCl₂ in the DNase I buffer.

An ATPase activity tracked with the A₃₅₀ from the Mono Q column (Fig. 7) and protein visible on the SDS gel, that had characteristic gpA packaging and cos-cleavage complementation activity. In order to be more certain that this ATPase activity could be attributed to gpA, a fraction of the Mono Q peak was subjected to heparin chromatography. ATPase activity tracked with gpA as visualized on an SDS gel, as did its packaging and cos-cleavage activity (Fig. 11). The UV absorbing material which eluted from fractions 4 to 13 was not protein (data not shown). The ATPase activity of gpA (Kₐ₅, 68 μM; turnover number, 62 min⁻¹) was very similar to that of terminase, and was stimulated 10–14-fold by both cos- and non cos-containing DNA.

It is known that after the staggered nicks are introduced in cosN, the annealed cohesive ends do not separate or melt spontaneously under physiological conditions since the hydrogen-bonded region is 12 base pairs in length. Terminase possesses a DNA helicase-like activity which can separate these ends in an ATP hydrolysis-dependent manner (Higgins et al., 1988).
holoenzyme and both subunits prepared as outlined above were assayed for helicase activity and the results are presented in Fig. 12. In this assay, linear mature λ Charon 8 DNA has been reduced to discrete fragments by a restriction endonuclease but the cosN single-strand ends are annealed unless the DNA is heated and fast cooled (lanes 1 and 2). Terminase promotes separation of the strands without heating (lane 3) and under conditions which do not support endonuclease activity (Rubinchik et al., 1994a), suggesting that this helicase-like activity is independent of the cos-cleavage reaction. This activity was not stimulated by IHF (lane 4). Neither BSA (lane 2), gpNu1 (lane 11), nor IHF (lane 12) demonstrated any similar activity. However, gpA at a comparable concentration was just as effective in promoting strand separation as the holoenzyme (lane 7). These results suggest that the DNA helicase activity domain of terminase resides in the gpA subunit. Neither gpA nor terminase displayed any activity in the absence of ATP (data not shown) nor in the presence of ATPγS (lanes 6, 8, and 10), confirming that this activity of gpA and terminase required ATP hydrolysis, as reported by Higgins et al. (1988).

Since three of terminase's major activities, i.e. cosN-cleavage, ATPase, and DNA helicase appeared to be functions of gpA, DNA packaging was also tested. The gpA concentration dependence for in vitro packaging is presented in Fig. 13. GpA at concentrations up to 3700 nM failed to exhibit any packaging ability except in the presence of a complimentary gpNu1 source.

We had consistently observed that the activities of freshly prepared gpA crude extracts were very labile at 42 °C, but were to some extent stabilized upon mixing with gpNu1 extracts (Davidson et al., 1991). In order to determine which of the several gpA activities was temperature-sensitive, the experiments depicted in Fig. 14 were performed. Terminase, gpNu1, and gpA were separately kept at 42 °C for various times and then tested for ATPase, helicase, packaging, and cos-cleavage. For the subunits, each heated sample was mixed with its unheated counterpart before assay. In addition, the two subunits were also heated together. For ATPase and helicase activities,
Bacteriophage λ Terminase Subunits

FIG. 12. DNA strand separation or helicase activity of terminase and its subunits. All reaction conditions were as described under "Experimental Procedures." S, migrating position of the two DNA fragments held together by the annealed cosN overhangs; PL and PS, positions of the large and the small fragments, respectively, that are produced when the annealed cosN overhangs are melted. Lanes 1 and 2, BSA, 0.1 mg/ml; lanes 3 and 5, 64 nM terminase; lanes 4 and 6, 64 nM terminase + 30 nM IHF; lanes 7 and 8, 132 nM gpA; lanes 9 and 10, 64 nM gpA + 132 nM gpNul; lane 11, 500 nM gpNul; lane 12, 60 nM IHF. In lanes 5, 6, 8, and 10, the reactions contained 12 nM ATP-S; 12 nM ATP was present in all the other reactions. Lane 1, the reaction mixture was heated at 65 °C for 5 min and fast cooled after the incubation; all other reactions were not heated.

FIG. 13. gpA concentration and in vitro packaging. Packaging reactions were as described under "Experimental Procedures." Solid circles indicate packaging reactions to which a 2-fold excess of gpNul was added to complement the amount of gpA indicated; solid squares indicate reactions to which only gpA was added.

which are completely stable in all the proteins at room temperature for at least 30 min, heating at 42 °C for 20 min resulted in less than a 5-fold reduction not only for the terminase (Panel B) but for gpA (Panels A and D) as well, whether or not gpNul was present (Panels A and D). (The ATPase activity of gpNul was not tested in this series of experiments.) However, while the packaging and cos-cleavage activity of terminase were reduced by approximately 100-fold in 10 min (Panel B), for gpA these activities were almost completely abolished in less than 5 min (Panel A). The presence of gpNul during the treatment did significantly protect the cleavage ability of gpA and also to a lesser extent, its packaging complementing ability (Panel D). At 52 °C (data not shown), the ATPase and helicase of terminase were slightly more labile than at 42 °C, while those of gpA were more affected, but still measurable after 10 min. Closer analysis revealed that the ATPase of both proteins was slightly more stable than helicase. The presence of gpNul during the treatment protected gpA to a significant extent. Cleavage and packaging activity, however, were completely destroyed within 2 min at 52 °C for both proteins. These results suggest that ATPase and helicase may reside in a distinct domain(s) different than those of cos-cleavage and packaging.

An interesting outcome of these experiments can be seen in Fig. 14, Panel C. When gpNul was heated by itself and then added to unheated gpA in a DNA packaging assay, its ability to complement was reduced more than 400-fold in 20 min. This effect was more pronounced at 52 °C (data not shown). However, when cutting was measured, no effect of heating gpNul by itself was observed at either temperature. These observations indicate that different domains of gpNul, with differing thermal stabilities, contribute to the packaging and endonuclease activities of the holoenzyme.

After several weeks of storage at ~70 °C, some samples of purified gpA appeared to have a slightly higher mobility in SDS-PAGE relative to various molecular weight standards. This suggested that some breakdown might be occurring and subsequent preparations were carried out in the presence of leupeptin, pepstatin, bestatin, and benzamidine hydrochloride, in addition to phenylmethylsulfonyl fluoride. However, certain aliquots of the peak from the Mono Q column still showed some diminution in size. The sequence of the first 30 amino-terminal residues was determined and in these samples clipping of the first 12 amino acids had occurred. A similar analysis was carried out on two more independent preparations where clipping was suspected and as before, the gpA had been clipped between arginine 12 and histidine 13. When some aliquots of a subsequent preparation also showed clipping, analysis revealed that it had occurred between leucine 22 and phenylalanine 23. Clipping has been observed for protein isolated from both expression vectors used here. The clipped gpA proteins still retained their endonuclease, ATPase, and helicase activities. With preparations which had lost 22 residues, however, activities that required association with gpNul, such as packaging and endonuclease at low protein concentrations, were reduced approximately 5-fold. Several terminase preparations prepared in the exact same manner as gpA were electrophoresed in denaturing gels, and the gpA band sequenced; no breakdown was ever observed. These results indicate that the amino terminus of gpA is extremely labile in solution when not associated with
gpN1. The clipping sites observed above do not appear to correspond to any known proteases. When either clipped or non-clipped preparations of gpA were incubated with a variety of pure proteins, no breakdown of the latter could be detected (data not shown).

**DISCUSSION**

**Bacteriophage λ terminase** is a complex multifunctional, multisubunit protein whose detailed structure remains to be determined. Its wide range of activities dictates the necessity of several kinds of molecular, biological, and enzymological analyses. Some of these determinations, like DNA packaging, must still be carried out in crude extracts of bacteria expressing the wild type genes; others, like ATPase or DNA helicase can only be reliably assayed with purified proteins because of the multiplicity of similar enzymes in the host bacteria. Furthermore, since terminase is composed of two different subunits, experiments with the holoenzyme alone cannot easily contribute to our understanding of how the various activities are organized, or the location of their domains. Most of our earlier information about the holoenzyme was derived from analysis of terminase isolated from induced lysogens (Gold and Becker, 1983). However, the latter could only be obtained by a very laborious and time-consuming multi-step procedure which started with nearly 5 kg of frozen bacteria and yielded only a few hundred micrograms of partially pure enzyme. The separation and cloning of the Nul and A genes, and the construction by recombinant DNA techniques of plasmids which could express great quantities of holoenzyme and subunit proteins were major breakthroughs which made many experiments possible for the first time (Chow et al., 1987; Keefer, 1987; Muraldo et al., 1987). We were able to isolate large amounts of essentially homogeneous gpN1 but these preparations were highly aggregated and required several fractionation steps (Parris et al., 1988). The isolation of a large number of spontaneous mutations in the terminase genes (Davidson et al., 1991) and the relative ease with which more could be made, made it obvious that a detailed study of the activity domains of terminase would require the development of new and better purification protocols. The first part of this paper describes our efforts to solve these problems and establish routines to facilitate our goals.

The procedures outlined above are rapid, reproducible, simple, and can yield milligram quantities of almost pure, nucleic acid-free unaggregated, soluble terminase, gpA and gpN1 from only 1 liter of bacterial culture (approximately 1 g wet weight of cell pellet). Although these methods were not primarily designed to achieve maximum yields, nor to produce the ultimate in purity, we find that with some minor modifications, they can be very easily adapted for large scale preparative purposes and even more refined homogeneity.

In the case of terminase and gpN1, at least where a comparison with previous preparations was possible, no significant differences in the qualitative or quantitative aspects of the various activities were observed. The variations measured could be the result of overproduction, or due to the much shorter times involved during these new fractionation manipulations. As we found earlier (Parris et al., 1988) more than 90% of the gpN1 protein expressed by these vectors was in the form of an insoluble particulate fraction. This did not appear to be the result of thermal induction since similar results were obtained when naldixic acid was used to induce transcription. (However, a single amino acid change in the protein, i.e. lysine 35 to glutamic acid, resulted in more than 50% of the total gpN1 becoming soluble (Yang, 1993)). The use of Sarkosyl to solubilize particulates in bacterial expression systems has been exploited successfully by Frankel et al. (1991) who also added this detergent to their lysis buffer. Studies on the tail-spike protein of bacteriophage P22 (Mitraki et al., 1991) suggest that aggregates of this type might be formed from specific, partially folded intermediates and it may be that in isolation from gpA, these gpN1 intermediates may not fold correctly. Nevertheless, the in vitro activities of gpN1 purified by the method described here are virtually identical to those previously found in protein purified from the soluble fraction of gpN1-expressing cells (Becker and Gold, 1988; Parris et al., 1988). The new method is more favorable for mechanism and structural studies because it yields primarily dimers as opposed to high molecular weight aggregates. Footprinting studies with previous aggregated fractions required 20-fold more protein (Shinder and Gold, 1988) than what is presently required to achieve full DNase I protection (Fig. 6).

Although in vivo the expression of gpN1 and gpA are not translationally coupled (Johnson et al., 1991) their genes do overlap in different reading frames in the phage. While this overlap has been eliminated in the construction of the overproducing terminase plasmids used here, it is highly likely that the induced pathways of each subunit are intimately intertwined as holoenzyme is formed, but when translated by themselves, the individual subunits assume some not completely "native" conformation. There are several observations which support this hypothesis. As reported here in confirmation of our previous findings (Parris et al., 1988), gpN1 forms insoluble particles within the cell when expressed in the absence of gpA. Certain preparations of gpA made from cells not expressing gpN1 undergo what appears to be spontaneous clipping at their amino terminus. No breakdown of gpA has ever been observed in any of our holoenzyme preparations even though the latter were derived from the same bacterial strain using virtually identical buffers, chromatography protocols, and storage conditions. Even in fresh crude extracts it has never been possible to combine the two subunits to always achieve levels of packaging or cos-cleavage attaining the levels of crude holoenzyme. In addition, some of the data presented above and also in the accompanying papers (Rubinich et al., 1994a, 1994b) which present detailed analysis of gpA's endonuclease, ATPase, and DNA helicase activities, clearly demonstrate that the purified subunits, when expressed in isolation from one another, cannot always be combined in complementation assays to achieve holoenzyme-like properties. Whether formation of holoenzyme is a spontaneous outcome of inter-related folding intermediates, or whether some active process involving host factors or chaperonins is taking place is currently being investigated. In the holoenzyme, the subunits are tightly coupled and cannot be separated by high ionic strength, strong ion-exchange chromatography, or hydrophobic interaction chromatography. We have been able to disrupt terminase only with guanidinium HCl or SDS. This suggests that the association of gpN1 and gpA is normally driven by specific forces which may require uniquely folded conformations which are unlikely to be realized in vitro. Thus, the inability of the subunits to fully complement in vitro even when in crude extracts may be due to the extreme aggregation of gpN1, which is always observed with these expression vectors, or to the improper folding of the gpA amino-terminal domain. Indeed, when the subunits of the purified holoenzyme are separated in guanidinium HCl, the small subunit, gpN1, can be renatured to a functional form which retains all of its in vitro activities. On the other hand, functional gpA cannot be recovered from this treatment, which suggests that this complex multidomain protein requires external assistance to achieve correct folding conformation. Nevertheless, the purified subunits of the holoenzyme can reassociate in vitro to a substantial extent, and the optimal gpN1 to gpA ratio for packaging was found to be 2:3:1 and for cutting
Bacteriophage λ Terminase Subunits

1–21, similar to the composition of holoenzyme.

The isolation and characterization of the gpA subunit has revealed somewhat surprisingly perhaps, that of 4 of the major biological/biochemical activities of terminase, i.e. DNA packaging, cos-cleavage, ATPase, and helicase, the latter three can be almost completely accounted for by gpA. As we show in the accompanying papers (Rubinchik et al., 1994a, 1994b) the kinetic parameters, nucleotide requirements, nick site specificity, and other properties exhibited by gpA are under certain in vitro conditions very similar to those of the holoenzyme. This discovery should facilitate future experiments which attempt to probe the mechanism and inter-relationships of the various activity domains of terminase.

The purification procedure outlined above yielded gpA that was predominantly a monomer in solution, but a tendency for dimerization was evident at certain protein concentrations. In native gel electrophoresis at higher protein concentrations, we have found that the predominant species of gpA migrated in somewhat surprisingly perhaps, that of 4 of the major conditions very similar to those of the holoenzyme. This discovery should facilitate future experiments which attempt to probe the mechanism and inter-relationships of the various activity domains of terminase.

The purification procedure outlined above yielded gpA that was predominantly a monomer in solution, but a tendency for dimerization was evident at certain protein concentrations. In native gel electrophoresis at higher protein concentrations, we have found that the predominant species of gpA migrated in two positions, indicative of molecular weights of 80,000 and 158,000, respectively. Because there is a putative "bZip" DNA binding motif in gpA containing a leucine zipper (Davidson and Black, 1988), we might have expected these preparations to have a much higher proportion of dimeric molecules. However, dimerization may only proceed after DNA binding has first taken place. The available evidence implies that at least two terminase protomers are required for cutting and packaging in vitro (Becker et al., 1977; Rubinchik et al., 1994a). Although we have been able to show that gpA can efficiently bind to various DNA molecules whether or not they contain cos, the footprinting experiments shown in Fig. 9 could not detect any protection of specific residues in cosN. It may be that the gpA-cosN interaction does not have to be very strong or specific because normally gpA exists only as a subunit of terminase which is already bound to DNA sites in the immediate vicinity by the action of gpNu1. This is apparent in Fig. 10 where the terminase footprint clearly shows protection of cosN.

Our initial impressions that some activities of gpA were thermodinlaye were confirmed by the experiments in Fig. 14. It seems that the ATPase and helicase domain transformation(s) are fairly heat stable, in contrast to those of cleavage and packaging.

The occasional loss of residues from the amino terminus of gpA purified from the overproducing strains does not appear to be a result of protease action either by gpA itself or some contaminant, and the mechanism of this clipping remains obscure. Nevertheless, the various enzymatic activities of gpA described above do not appear to entirely rely on the presence of at least its first 22 amino acids. We are currently preparing by genetic, chemical, and enzymatic procedures other fragments of gpA in order to test them and map the activities of the protein.

As seen in Fig. 13, gpA was not by itself capable of promoting DNA packaging. Since gpNu1 can also not package by itself, we are left with the problem of locating the domain(s) responsible. It has generally been assumed that the packaging "engine" is resident in gpA, mainly because of a variety of indirect evidence and also inferences drawn from other phage systems. It is clear that the prohead capture domain resides in gpA, probably very near to the carboxyl terminus (Sipp and Feiss, 1992). Also, our earlier work with a temperature-sensitive mutant (Becker et al., 1977) showed that gpA activity was required throughout the entire packaging cycle, including translocation and full head completion (Perucchetti et al., 1988). In some phage systems, the small gpNu1 subunit equivalent is not necessary for packaging mature DNA, for example, in T4, where this reaction can be carried out by the large subunit, gp17, by itself (Hsiao and Black, 1977; Rao and Black, 1988). It has also been suggested in other systems, notably d29 (Turnquist et al., 1992), that the packaging motor is actually the connector at the portal vertex of the prohead and that the terminase large subunit's ATPase provides the energy required for the translocation.

Although the genetic and enzymatic evidence now clearly establish that gpA is a cosN-specific endonuclease, mutational analysis did not make this conclusion obvious. Several different mutations in Nu1 (Davidson et al., 1991; Willis, 1992; Yang, 1993) result in the expression of holoenzymes which cannot carry out cos-cleavage even though they contain a wild type gpA subunit. For example, a 4-amino acid insertion in the o2 helix of the putative α helix turn helix DNA binding fold of gpNu1 (Kypr and Mrazeck, 1986) creates a holoenzyme which appears to footprint normally to cosB (Shinder and Gold, 1988), but which displays little if any detectable cos-nicking or cos-cleavage. DNA packaging with this mutant terminase was only reduced 400-fold. On the other hand, a similar insertion in the COOH-terminal half of gpNu1 had only a slight affect on the endonuclease properties of the holoenzyme where it reduced packaging efficiency by a factor of more than 103 and appeared to completely abolish binding. These and similar observations strongly imply that one of the roles of gpNu1 is to, in an as yet unknown manner, modulate the activity of gpA and perhaps to activate its packaging domain to translocate the DNA into the prohead. The experiment shown in Fig. 14 demonstrates that in whichever way gpNu1 cooperates with gpA to promote packaging, this function is clearly distinct from its other activities since it is much more temperature sensitive. We are currently attempting to locate this gpNu1 domain.

Our findings in this report demonstrate that gpNu1 is not essential for cleavage at cosN even though it is clear that gpNu1 does, in a manner which is as yet not entirely clear, modulate gpA's activity both qualitatively and quantitatively. The experiments described in Fig. 8 suggest that even cosB may not be entirely essential for cleavage at cosN by either gpA or terminase. The results with terminase in Fig. 8 confirm earlier findings both in vivo and in vitro by Miwa and Matsubara (1982, 1983), Higgins et al. (1988), and Xu (1989), who found that the absence of all four R sites in cosB did not completely eliminate cos-cleavage nor even packaging. It should be emphasized that the results in Fig. 8 are reflective of yields of the reaction under optimal in vitro conditions and thus reveal the substrate capacity of the different DNA fragments. Kinetic analysis, however, did show that depending on the salt concentration, the initial rate of cleavage is decreased by approximately 3-10-fold in the absence of cosB. This value corresponds well with that determined by Miwa and Matsubara (1983) and Xu (1989). Our observations (Willis, 1992) that terminase can efficiently cut a plasmid with the cosB R1, R2, and R3 sites of phage 21 also suggest that the substrate or phage specificity of cos-cleavage may not be ascribable solely to the interaction of gpNu1 and/or gp1 to their respective binding sites in cosB (Becker and Murialdo, 1990). This result is not that surprising in light of the fact that gpA by itself can promote cosN cleavage and that cosN is similar in these two phases. The ability of overproduced, purified terminase to cleave a 21 cosI substrate as efficiently as that of λ (Willis, 1992) is not in agreement with the results of Feiss and Widner (1982), who found that with crude extracts, λ terminase could not eliminate the 21 cosB substrate. Although this discrepancy could be due to inherent differences between wild type and overproduced holoenzyme, or between crude extracts and a concentrated highly purified protein, it should be pointed out that the in vitro conditions optimal for the endonuclease activities of gpA and holoenzyme employed in this work are not conducive to high fidelity of cosN-nicking (Rubinchik et al., 1994a). The experiments of Feiss and Widner (1982) were carried out under what we will
show are very restrictive conditions promoting high specificity; these conditions appear to resemble those probably prevailing in vivo.

In conclusion, we have developed rapid and simple methods for purifying considerable amounts of the bacteriophage λ terminase and its subunits from small volumes of bacterial cultures. The isolated gpA subunit cannot by itself promote DNA packaging unless gpNul is present. However, it appears that the cos-cleavage, ATPase, and DNA-helicase activities of the holoenzyme can be almost completely accounted for by gpA.

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REFERENCES

Becker, A., and Gold, M. (1988) J. Mol. Biol. 199, 219–222
Becker, A., and Murialdo, H. (1990) J. Bacteriol. 172, 2819–2824
Becker, A., Marko, M., and Gold, M. (1977) Virology 76, 291–306
Berg, J. M. (1986) Science 232, 485–487
Chow, S., Dubb, E., and Murialdo, H. (1987) Gene (Amst.) 60, 277–289
Davidson, A., and Gold, M. (1992) Virology 199, 21–30
Davidson, A., Yu, P., Murialdo, H., and Gold, M. (1991) J. Bacteriol. 173, 5096–5096
Feiss, M. (1986) Trends Genet. 2, 100–104
Feiss, M., and Becker, A. (1985) in Lambda II (Hendrix, R. W., Roberts, J. W., Stahl, F. W., and Weinberg, R. A., eds) pp. 305–330, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Feiss, M., and Widner, W. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 3498–3502
Frankel, S., Sohn, R., and Leinwald, L. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 1192–1196
Gold, M., and Becker, A. (1983) J. Biol. Chem. 258, 14619–14625
Gold, L., Pribnow, D., Schneider, T., Schindelin, S., Singer, B.S., and Storms, G. (1981) Annu. Rev. Microbiol. 35, 365–403
Guo, P., Peterson, C., and Anderson, D. (1987) J. Mol. Biol. 197, 229–236
Higgins, R. R., Lucko, H. J., and Becker, A. (1988) Cell 54, 765–775
Hissin, C. L., and Black, L. W. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3652–3656
Jenkins, W. T. (1991) Anal. Biochem. 194, 136–139
Johnston, R. P., Pickett, S. C., and Barker, D. L. (1990) Electrophoresis 11, 355–360
Johnson, G., Widner, W., Xin, W., and Feiss, M. (1991) J. Bacteriol. 173, 2733–2738

Kallwass, H. K. W., Luyten, M. A., Parris, W. Gold, M., Kay, C., and Jones, J. B. (1992) J. Am. Chem. Soc. 114, 4551–4557
Keeler, C. L., Jr. (1987) The Cloning, Overproduction and Expression of the Terminase Genes from Bacteriophage Lambda, Ph.D. Thesis, University of Maryland, Baltimore, MD
Kypr, J., and Mrozek, J. (1986) J. Mol. Biol. 191, 139–140
Laemmli, U. K. (1970) Nature 227, 680–685
Layne, E. (1957) Methods Enzymol. 3, 447–453
Le Gendre, N., and Matsuura, P. (1980) In A Practical Guide to Protein and Peptide Purification for Microsequencing (Matsudaika, P., ed) pp. 51–69, Academic Press Inc., Orlando, FL
Miller, G., and Feiss, M. (1986) J. Mol. Biol. 183, 246–249
Mitrakd, A., Fane, B., Haase-Pettingell, C., Sturtevant, J., and King, J. (1991) Science 253, 54–58
Miwa, T., and Matsuura, K. (1982) Gene (Amst.) 20, 265–277
Miwa, T., and Matsuura, K. (1985) Gene (Amst.) 24, 199–206
Murialdo, H. (1991) Annu. Rev. Biochem. 60, 125–153
Murialdo, H., Davidson, A., Chow, S., and Gold, M. (1987) Nucleic Acids Res. 15, 119–140
Nash, H. A., Robertson, C. A., Flamm, E., Weisberg, R. A., and Miller, H. J. (1987) J. Bacteriol. 169, 4124–4127
Parris, W., Davidson, A., Keeler, C. L., Jr., and Gold, M. (1988) J. Biol. Chem. 263, 8413–8419
Perucchini, R., Parris, W., Becker, A., and Gold, M. (1988) Virology 165, 103–114
Rao, V. B., and Black, L. W. (1986) J. Mol. Biol. 200, 475–488
Reyes, O., Gottasman, M., and Ashby, S. (1979) Virology 94, 499–498
Rubinchik, S., Parris, W., and Gold, M. (1994) J. Biol. Chem. 269, 13575–13585
Rubinchik, S., Parris, W., and Gold, M. (1994) J. Biol. Chem. 269, 13586–13593
Snyder, G., and Gold, M. (1988) J. Virol. 62, 367–362
Sigg, J., and Feiss, M. (1992) J. Bacteriol. 174, 850–856
Smith, M. P., and Feiss, M. (1993) J. Bacteriol. 175, 2393–2399
Tomko, M., and Catalano, C. (1993) J. Biol. Chem. 268, 3065–3068
Turqueait, S., Simon, M., Egelman, E., and Anderson, D. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 10479–10483
Vinson, C. R., Sigler, P. B., and McKnight, S. L. (1989) Science 246, 911–916
Wills, R. C. (1992) Mutational Analysis of Bacteriophage Lambda Terminase: Possible Role of Nul Subunit in Endonucleolytic Cleavage of CoS. Ph.D. Thesis, University of Toronto
Wo, W. P., Christiansen, S., and Feiss, M. (1988) Genetics 119, 477–484
Yang, Y.-C. (1986) Site-directed Mutagenesis and Purification of gpNul, the Small Subunit of Bacteriophage Lambda Terminase, Ph.D. Thesis, University of Toronto
Yandofsky, C., and Ito, J. (1966) J. Mol. Biol. 21, 313–334
Xu, S. (1989) Bacteriophage Lambda DNA Packaging and Injection, Ph.D. Thesis, University of Iowa