Role of gpFI Protein in DNA Packaging by Bacteriophage λ†

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ABSTRACT: One of the final steps in the assembly of bacteriophage λ is the excision of a single genome from a concatemeric DNA precursor and insertion of this monomer into a preformed capsid. Terminase enzymes are common to all of the double-stranded DNA phages, and in λ this enzyme is responsible for both excision of a genome monomer from the concatemer and its insertion into the pro-capsid. We have previously demonstrated that the endonuclease activity of λ terminase (*cos*-cleavage) was stoichiometric with enzyme and postulated that this was due to formation of a stable, postcleavage enzyme•DNA intermediate (complex I) (Tomka & Catalano, 1993b). Bacteriophage λ gpFI protein is required for efficient assembly of the virus, and current models suggest that this protein increases the rate of pro-capsid binding to complex I. We show here that gpFI markedly stimulates *cos*-cleavage by λ terminase, even in the absence of viral pro-capsids. Importantly, the observed increase in nicking activity did not result from an increase in the *rate* of *cos*-cleavage, but rather by an increase in *turnover* by the enzyme. These data suggest that gpFI destabilizes complex I, thus allowing terminase release from *cos* and catalytic turnover by the enzyme. The implications of these results with respect to terminase assembly onto viral DNA, nicking of the duplex, and subsequent translocation during packaging are discussed.

Assembly of an infectious virus requires the interaction of a variety of proteins of both viral and host origin. Bacteriophage λ is assembled from preformed capsids, or proheads, fully assembled tails, and 48.5 kilobase (kb)[1] genomes that are excised from a concatemeric DNA precursor (Becker & Murialdo, 1990; Feiss, 1986; Murialdo, 1991). Work currently underway in our laboratory focuses on the biochemical and biophycial properties of DNA packaging in bacteriophage λ, and specifically the structure and function of the multiprotein complex responsible for this process.

Packaging of the λ genome requires condensation of DNA into the confined space within the viral capsid. The "engine" driving packaging is composed, at least in part, of a heteromultimeric enzyme known as terminase (Feiss & Becker, 1983). This enzyme, derived from the λ gene products of *A* and *Nu1* (Figure 1), possesses multiple catalytic activities including site-specific nuclease, DNA helicase, DNA translocase, and ATPase activities (Becker & Murialdo, 1990; Murialdo, 1991; Tomka & Catalano, 1993a,b). ATP, *Escherichia coli* integration host factor (IHF), and other phage proteins (gpFI, gpD, gpW, gpFII, etc.) are also required for the formation of a stable, DNA-filled capsid, but in many cases their roles remain ill-defined (Feiss & Becker, 1983).

The packaging pathway for λ has been described (Becker & Murialdo, 1990; Feiss, 1986; Feiss & Becker, 1983; Murialdo, 1991) and proceeds as follows: (i) The terminase subunits (gpA, gpNu1) and IHF assemble onto a multielement DNA packaging site (*cos*), forming a multiprotein nicking complex.[2] Terminase introduces staggered nicks into the duplex 12 bases apart and separates the nicked, annealed strands, yielding the enzyme bound to the mature left end of the λ genome (T•D_L, Figure 1). (ii) This stable intermediate, known as complex I, binds to a preformed capsid, yielding a packaging intermediate known as complex II, and insertion of DNA into the capsid ensues. Energy for the terminase-mediated translocation along the duplex is presumably provided by ATP hydrolysis. (iii) Upon encountering the next downstream *cos* (the end of the genome), the translocating terminase complex stops and again introduces staggered nicks into the duplex. Strand separation simultaneously results in formation of the 12 base single-stranded right end of the mature λ genome (D_R) and release of the DNA-filled head from the enzyme•DNA complex.

Bacteriophage λ gpFI protein plays an important, but ill-defined, role in the packaging process (Becker et al., 1988; Benchimol & Becker, 1982; Feiss & Becker, 1983; Murialdo et al., 1981a). This protein is expressed in large amounts (≈10^5 copies/cell, ≈150 μM) in the phage-infected cell but

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[1] Abbreviations: BSA, bovine serum albumin; β-ME, 2-mercaptoethanol; bp, base pair(s); *cos*, cohesive end site (the junction between individual genomes in immature concatameric λ DNA); IHF, *E. coli* integration host factor; IPTG, isopropyl thiogalactoside; kb, kilobase(s); PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonyl fluoride; pol II, RNA polymerase II; SDS, sodium dodecyl sulfate; TBE, Tris, borate, EDTA buffer.

[2] The nature of the nicking complex with respect to the protein subunit stoichiometry (gpA_x•$gpNu1_y$•IHF_z) remains ambiguous. Moreover, the protein composition likely changes in the transition from the initial nicking complex to the translocating complex and finally to the terminal nicking complex. We will use the term nicking complex to specifically refer to the multiprotein complex which initially assembles at *cos* and is responsible for nicking of the duplex and strand separation.

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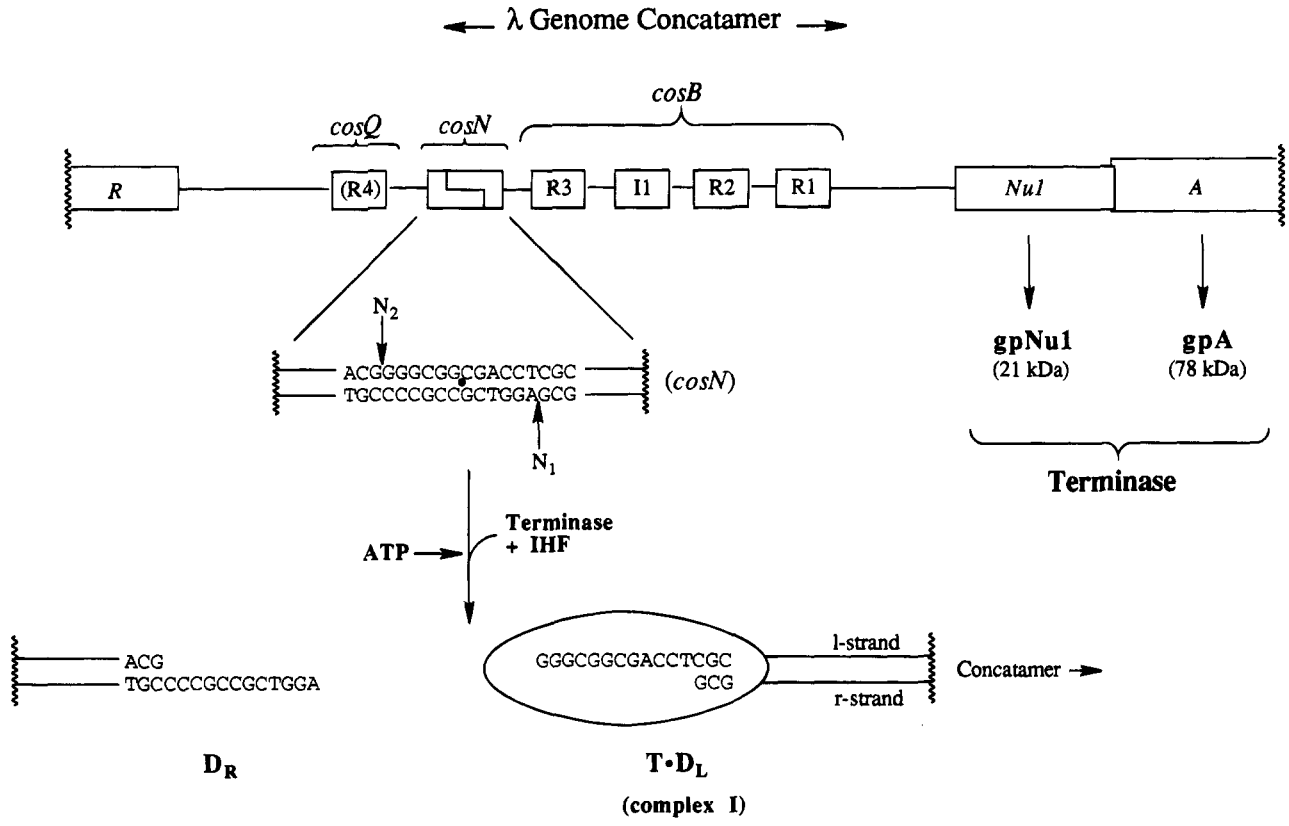


FIGURE 1: Phage λ terminase and the *cos*-cleavage reaction. The region of the λ genome spanning *cos* is shown at the top of the figure. The entire *cos* sequence includes R4-*cosN*-R3-I1-R2-R1 elements. The positions of *cosQ*, *cosN*, and *cosB* are indicated as are *Nu1* and *A*, the genes coding for terminase subunit polypeptides (gpNu1 and gpA). The DNA sequence of *cosN* is shown in the center of the figure with the center of symmetry within *cosN* indicated with a (•). Symmetric nicking by terminase (the TER reaction) normally occurs at N_1 and N_2, thus yielding D_R and D_L, the right and left ends, respectively, of the mature λ genome.

is not found in the mature virion particle (Feiss & Becker, 1983; Murialdo & Siminovitch, 1972). Infection of *E. coli* with λ *FI*⁻ phage results in the accumulation of immature (concatemeric) λ DNA and empty, unexpanded proheads. This morphology resembles infection with λ *A*⁻ and λ *Nu1*⁻ phage and indicates a failure to package DNA (Benchimol & Becker, 1982; Hohn et al., 1975). Terminase, proheads, and concatemeric λ DNA isolated from λ *FI*⁻ phage-infected cells are all competent to assemble phage *in vitro* (Benchimol & Becker, 1982; Hohn et al., 1975), which suggests that gpFI is required for proper coupling of the three components for efficient genome packaging. *FI*⁻ mutations are leaky, however, indicating that this protein is not strictly required for phage assembly (Feiss et al., 1988; Murialdo et al., 1981b). Current models suggest that gpFI protein stimulates the rate of complex I binding to proheads and thus increases the overall rate of DNA packaging by terminase (Becker et al., 1988).

We have previously characterized the kinetics of *cos*-cleavage by purified λ terminase and have shown that in the absence of viral proheads, the extent of DNA cutting was stoichiometric with the enzyme added to the reaction mixture (Tomka & Catalano, 1993b). This was consistent with the formation of a stable packaging intermediate (complex I), and we postulated that the addition of viral proheads to the reaction mixture would allow catalytic turnover as packaging of the DNA was attempted. During the course of our investigation into the effect of purified λ proheads on *cos*-cleavage by λ terminase *in vitro*, we noted that purified gpFI protein directly stimulated this reaction. Furthermore, we found that the endonuclease reaction was stimulated by gpFI even in the absence of viral proheads. We present here experiments that suggest a new role for gpFI in the DNA packaging pathway of phage λ. The implications of these results with respect to current models for λ phage assembly are discussed.

EXPERIMENTAL PROCEDURES

Materials and Methods. Tryptone, yeast extract, and agar were purchased from DIFCO. Restriction enzymes were purchased from Promega. Mono-Q HR5/5 FPLC columns and phenyl-Sepharose chromatography resins were purchased from Pharmacia. Sephadex G100, nucleoside triphosphates, and ampicillin were purchased from Sigma Chemical Co. All other materials were of the highest quality commercially available.

Large-scale bacterial cultures were performed using a New Brunswick Scientific 14 L Microferm fermentor. Smaller cultures were grown in shaker flasks and utilized a New Brunswick Scientific series 25 incubator−shaker. Protein purifications utilized a Pharmacia FPLC system which consisted of two P500 pumps, a GP250-plus controller, a V7 injector, and a Uvicord SII variable-wavelength detector. Densitometer analysis of SDS−PAGE gels utilized a Molecular Dynamics laser densitometer and the ImageQuant analysis package. UV−visible absorbance spectra were recorded on a Hewlett-Packard HP8452A diode array spectrophotometer. CD spectra were recorded on an Aviv Model 62D5 circular dichroism spectropolarimeter as described previously (Tomka & Catalano, 1993b).

Bacterial Strains, DNA Preparation, and Protein Purification. The plasmid pWP14 was purified from a cell line kindly provided by M. Gold (University of Toronto, Ontario, Canada) using Qiagen mega-prep DNA columns. Linearization with *Sca*I and quantitation of DNA concentration were performed as previously described (Tomka & Catalano, 1993b). Phage λ terminase was purified from AZ1930 (generously provided by H. Murialdo, University of Toronto, Ontario, Canada) as described previously (Tomka & Catalano, 1993b). *E. coli* integration host factor was purified from HN880 (a kind gift of H. Nash, National Institutes of Health, Bethesda, MD) by the method of Nash et al. (1987). Phage λ proheads were isolated from R594 (λ $cI_{857}A^{-}K^{-}S^{-}$) cells (a generous gift of D. Anderson) and purified by sucrose gradient centrifugation as described by Guo et al. (1986). All of our purified proteins were homogenous as determined by SDS−PAGE. Protein concentrations were determined by the method of Bradford (1976).

Purification of Phage λ gpFI Protein. HW33, an *E. coli* strain which overexpresses the gpFI protein, was kindly provided by M. Feiss (University of Iowa, Iowa City, IA). An overnight culture of HW33 (40 mL) was used to inoculate 4 L of LB containing 25 μg/mL ampicillin, and the cells were grown at 37 °C with constant stirring (200 rpm paddle speed) and aeration (3000 cm^3/min) until an optical density of 0.4 (600 nm) was attained. IPTG was then added to a final concentration of 1.2 mM, and the cells were maintained at 37 °C for an additional 5 h and then harvested by centrifugation. Unless otherwise indicated, all subsequent steps were carried out at 0−4 °C.

gpFI protein was purified as described by Benchimol and Becker (Benchimol & Becker, 1982; Benchimol et al., 1982) with modifications. The cells were suspended in buffer A (20 mM Tris, pH 8, 1 mM EDTA, 7 mM β-ME, and 5% glycerol) containing 1 mM PMSF and lysed by sonication. Insoluble cellular debris was removed by centrifugation, and ammonium sulfate fractionation was performed directly on the clarified lysate as described by Benchimol and Becker (1982). The 30% AS pellet was resolubilized in a minimal volume of buffer A containing 25% AS, and the protein fraction was applied to a phenyl-Sepharose column (20 mL) equilibrated with the same buffer. The column was washed until the absorbance had returned to base line, and protein was then eluted with a 100 mL gradient to 0% AS. Fractions (4 mL) were collected and examined for the presence of gpFI protein by SDS−PAGE. gpFI eluted at ≈10% AS. The appropriate fractions were pooled, dialyzed against buffer A, and applied to a Mono-Q HR5/5 column equilibrated with the same buffer. The column was washed with buffer A until the absorbance had returned to base line, and the protein was then eluted with a 30 mL gradient to 700 mM NaCl. Each fraction (1 mL) was examined for gpFI protein by SDS−PAGE, and the appropriate fractions were pooled and dialyzed against buffer B (50 mM Tris, pH 8, 1 mM EDTA, 7 mM β-ME, 100 mM NaCl, and 5% glycerol). gpFI eluted at ≈400 mM NaCl. The protein sample (5 mL) was next applied to a Sephadex G100 superfine column (2.5 cm × 15 cm) equilibrated and run with buffer B. Fractions (4.5 mL) were examined for gpFI protein by SDS−PAGE, and the appropriate fractions were pooled and dialyzed against buffer A. The protein was concentrated on a Mono-Q HR5/5 column as described above. The appropriate fractions were pooled and dialyzed against buffer A containing 50% glycerol, and the concentrated protein was stored at −80 °C.

cos-Cleavage Assays. Unless otherwise indicated, the reaction mixtures (12 μL) contained 50 mM Tris-HCl, pH 9, 11 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP, 7 mM β-ME, and 7 mM spermidine. *Sca*I-linearized pWP14, IHF, terminase and phage λ gpFI protein were added as described in each individual experiment. The reaction was initiated with the addition of $MgCl_2$ and allowed to proceed at 37 °C. Aliquots (3 μL) were removed at the indicated times, and the reaction was quenched with the addition of 7 μL of stop buffer (7.5% ficol, 30 mM EDTA, 0.15% SDS, and 0.03% bromophenol blue). The DNA products were fractionated by 6% nondenaturing PAGE using TBE as the running buffer. Electrophoresis was continued at 20 mA for ≈30 min after the xylene cyanol dye marker had migrated off the end of the gel. The DNA bands were visualized by staining with ethidium bromide and quantitated using video densitometry and the ImageQuant image analysis program as previously described (Tomka & Catalano, 1993b). The kinetic parameters for *cos*-cleavage were obtained by nonlinear regression analysis of the experimental data as described previously (Tomka & Catalano, 1993a,b).

RESULTS

Purification of Bacteriophage λ gpFI Protein. We have purified gpFI protein to homogeneity using a modification of the protocol originally published by Benchimol and Becker (Benchimol & Becker, 1982; Benchimol et al., 1982). The overall yield of protein was 2.4 mg from 4 L of bacterial culture with a purity of >98% as determined by SDS−PAGE (Figure 2A). This represents a significant improvement over the previously published protocol where a recovery of 0.3 mg of 80−90% homogeneous protein was obtained from 3.6 L of cell culture (Benchimol & Becker, 1982). We were unable to determine an extinction coefficient for purified gpFI as the protein does not contain any tryptophan, tyrosine, or cysteine residues (Daniels et al., 1983). Consistent with previous studies which concluded that gpFI protein existed as a monomer in solution (Benchimol & Becker, 1982), no evidence of protein aggregation was observed in our purified protein preparation (data not shown). The circular dichroism spectrum for purified gpFI is shown in Figure 2B and is consistent with a protein composed of ≈15% α-helical and 35% β-sheet structures.

The gpFI Protein Stimulates cos-Cleavage by λ Terminase. Previous studies in our laboratory have demonstrated that *cos*-cleavage by terminase in the absence of proheads was limited by the quantity of enzyme added to the reaction mixture (Tomka & Catalano, 1993b). We suggested that stoichiometric cutting was due to the formation of complex I, a stable terminase·DNA intermediate in the packaging pathway ($T{\cdot}D_L$, Figure 1), and that the reaction kinetics were thus limited by the concentration of enzyme added to the reaction mixture. This posit predicted that addition of viral proheads to the reaction mixture should allow multiple catalytic turnovers as the enzyme translocated from *cos* to initiate DNA packaging. Under these conditions, the reaction kinetics would no longer be limited by enzyme concentration, but rather by the number of viable proheads added to the reaction mixture.

Under conditions of excess DNA substrate (multiple-turnover conditions), proheads indeed stimulated *cos*-cleav-

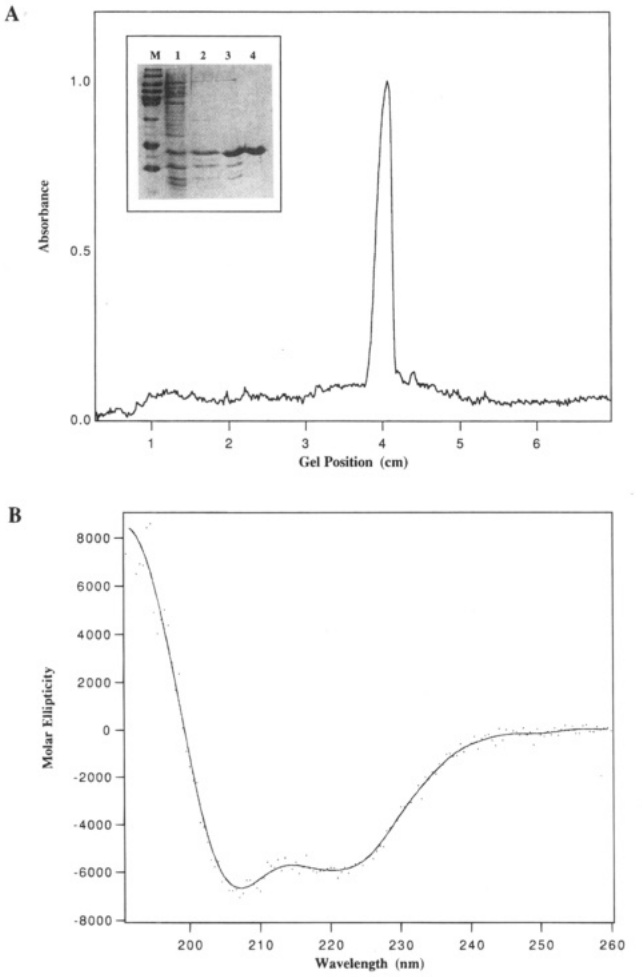


FIGURE 2: Purification of phage λ gpFI protein. Panel A: Densitometer scan of purified gpFI fractionated by SDS−PAGE (see inset, lane 4). Integration of the scan yielded a protein purity of greater than 98%. Inset: The various fractions obtained during the purification of gpFI protein were examined by 18% SDS−PAGE. Lanes: (M) protein molecular mass markers (Promega: 97.4 kDa, phosphorylase B; 66.2 kDa, bovine serum albumin; 55.0 kDa, glutamate dehydrogenase; 42.7 kDa, ovalbumin; 40.0 kDa, aldolase; 31.0 kDa, carbonic anhydrase; 21.5 kDa, soybean trypsin inhibitor; 14.4 kDa, lysozyme); (1) crude cell lysate; (2) pooled phenyl-Sepharose fractions; (3) pooled Mono-Q fractions; (4) pooled Sephadex G100 fractions (after concentration). Panel B: CD spectrum of purified gpFI protein. Molar ellipticity is expressed as degrees centimeter squared per decimole. Conditions utilized to obtain the spectra were as previously described (Tomka & Catalano, 1993b).

age (Table 1). The observed stimulation was not as great as we would have expected, however. Since phage λ gpFI protein has been postulated to increase the efficiency of prohead interactions with complex I (Becker et al., 1988), we reasoned that this phage protein might be necessary for efficient prohead utilization *in vitro*. Consistent with this prediction, gpFI stimulated *cos*-cleavage 2−3-fold under conditions of limiting enzyme (Table 1). To our surprise, however, the observed stimulation was independent of viral proheads. Importantly, addition of gpFI had only a modest effect on DNA nicking under single-turnover reaction conditions (excess enzyme), either in the presence or in the absence of viral proheads (Table 1).

The observed stimulation of *cos*-cleavage was specific as neither heat-inactivated gpFI protein nor albumin added to an equivalent concentration affected the reaction (not shown). Moreover, gpFI alone did not possess any detectable *cos*-specific endonuclease activity. The observed stimulation was further dependent on the concentration of gpFI added to the reaction mixture (Table 2).

Table 1: Effect of Proheads and gpFI Protein on *cos*-Cleavage[a]

[terminase] (nM)	[proheads] (nM)	[gpFI] (μM)	relative activity[b]
50	0	0	1.0
50	30	0	1.5
50	0	30	2.3
50	30	30	2.8
500	0	0	1.0
500	30	0	1.1
500	0	30	1.4
500	30	30	1.4

[a] Reactions were performed as described under Experimental Procedures with DNA and IHF each added to 100 nM. Terminase, purified proheads, and gpFI were added as indicated in the table. [b] A relative activity of 1.0 refers to the amount of DNA cut in 30 min in the absence of proheads and gpFI and corresponds to 5% and 29% of the input DNA in the presence of 50 and 500 nM terminase, respectively.

Table 2: Stimulation of *cos*-Cleavage Is Dependent on the Concentration of gpFI[a]

[gpFI] (μM)	% DNA cut
0	9
33	17
55	27

[a] Reaction conditions were as described under Experimental Procedures with DNA and IHF each added to 100 nM and terminase added to 50 nM. gpFI was added as indicated in the table and the amount of DNA cut at 30 min analyzed as described.

The gpFI Protein Stimulates the Extent, but Not the Rate, of cos-Cleavage. Under conditions of excess DNA substrate (multiple-turnover conditions), *cos*-cleavage was limited by the amount of terminase added to the reaction mixture, and a single catalytic turnover was observed (Figure 3A). Consistent with results previously obtained in our laboratory (Tomka & Catalano, 1993b), roughly 0.5 enzyme equiv of DNA was cut, suggesting that two terminase promoters were required to nick a single DNA substrate. Addition of phage λ gpFI protein to the reaction mixture dramatically increased the *extent* of *cos*-cleavage (16% → 100%).[3] The *rate* of the reaction was not significantly altered, however, with observed rates of 0.035 and 0.041 min^{-1} in the absence and presence, respectively, of gpFI. These data suggest that neither the rate of complex assembly nor the rate of nicking was significantly affected by gpFI. To test this directly, *cos*-cleavage in the presence of excess enzyme (single-turnover conditions) was examined. Under these conditions, addition

[3] We note that gpFI-mediated stimulation was greater in this experiment than that presented in Table 2 where the degree of stimulation was ≈2-fold and consistent with the results presented in Table 1 where similar reaction conditions were used. The experiment presented in Figure 3 utilized higher enzyme and DNA concentrations, conditions where assembly of complex I, the rate-limiting step in *cos*-cleavage, is expected to be faster [see Tomka and Catalano (1993b)]. The extent of *cos*-cleavage at 30 min is similar in the absence of gpFI in both experiments (≈10%, see Table 2 and Figure 3) since *turnover* by the enzyme does not occur. Addition of gpFI allows turnover by the enzyme which is faster with increased enzyme concentrations (increase in the rate-limiting step) and the observed degree of stimulation at 30 min is thus greater in Figure 3. Note that while the rate of complex I assembly is enhanced with elevated enzyme and DNA concentrations, it is *not* affected by gpFI (see Figure 3).

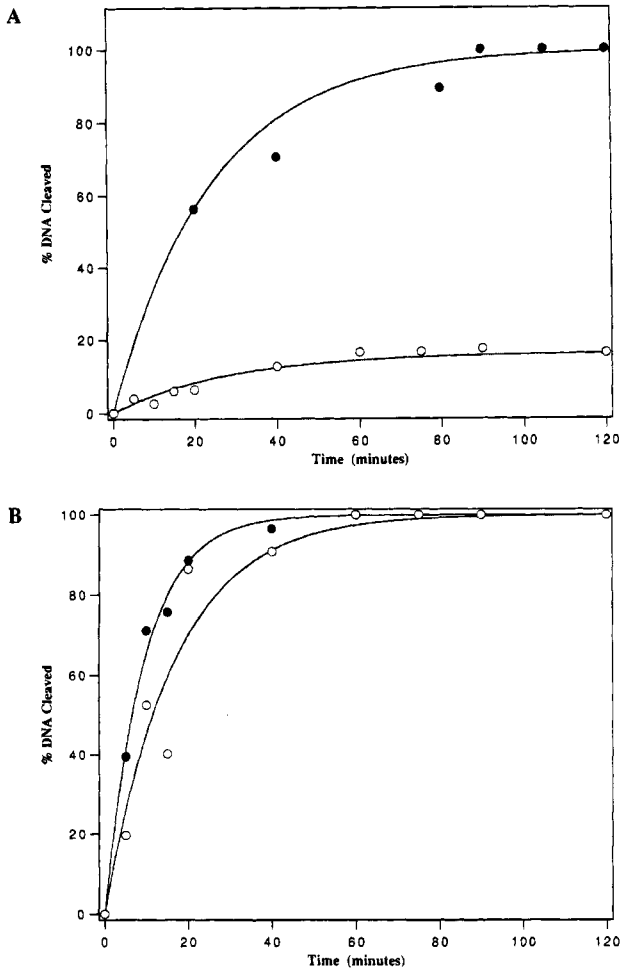


FIGURE 3: Phage λ gpFI protein stimulates the extent of *cos*-cleavage. The extent of DNA cleavage in the absence (○) or presence (●) of 30 μM gpFI. Panel A: Multiple-turnover (excess DNA substrate) catalytic conditions. The reaction conditions were as described under Experimental Procedures with terminase and DNA added to 100 and 500 nM, respectively. Panel B: single-turnover (excess enzyme) catalytic conditions. The reaction conditions were as described under Experimental Procedures with terminase and DNA added to 800 and 250 nM, respectively. Each data point represents the average of two separate experiments.

of gpFI to the reaction mixture only modestly affected the time course of the DNA nicking reaction (Figure 3B).

DISCUSSION

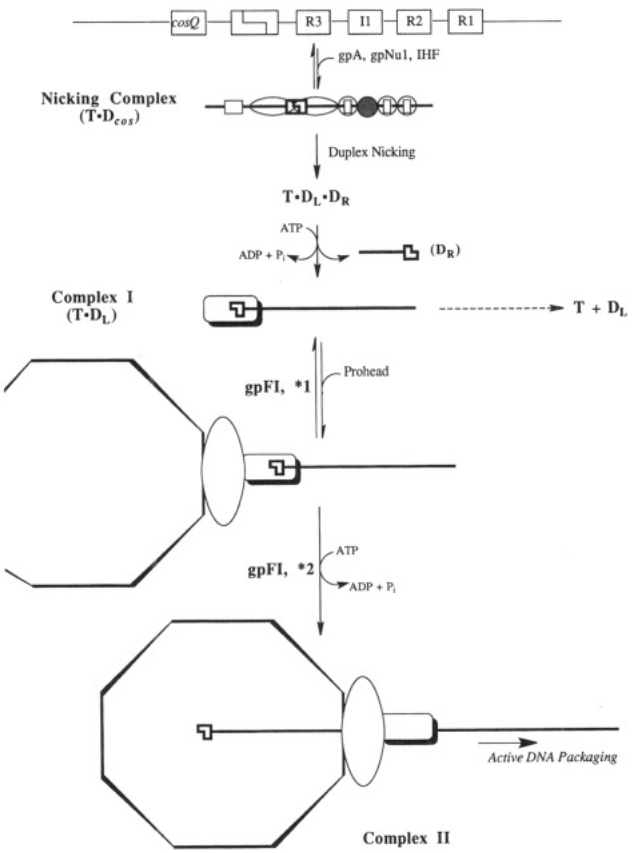
Packaging of λ DNA within a viral capsid proceeds through several intermediates derived from the interaction of both viral and host proteins with a concatemeric DNA substrate (Becker & Murialdo, 1990; Feiss & Becker, 1983; Murialdo, 1991). An early intermediate is a multiprotein complex referred to as complex I (see Figure 1). Complex I is quite stable, indicating strong bonding interactions between terminase and *cos*-containing DNA. Specific enzyme•*cos* interactions must be disrupted upon prohead binding, however, so that the terminase•prohead complex may translocate along the duplex during active packaging of the viral genome. The events leading from a protein complex which binds tightly and specifically at *cos* to one which releases this site but remains tightly bound to nonspecific DNA during packaging remain a mystery. We note that while these events may be a function of complex I itself, it is likely that the prohead, gpFI protein, or both trigger the transition leading from a stable DNA nicking complex to a mobile DNA packaging machine.

Currently favored models for the role of gpFI in λ assembly suggest that this phage protein stimulates prohead interactions with complex I, thus increasing the overall rate of DNA packaging (Becker & Murialdo, 1990; Becker et al., 1988; Murialdo, 1991). This model was based on *in vitro* phage assembly studies where complementing phage-infected cell extracts deficient in specific viral proteins were combined and their effect on complex I and complex II formation examined (Becker et al., 1988). These studies demonstrated that the gpFI protein stimulated complex II formation ≈16-fold but little affected the formation of complex I. The mechanism by which gpFI stimulated complex II formation was unclear, and several models were proposed for the requirement of gpFI in DNA packaging. Importantly, models which invoked events prior to prohead binding were not favored by these authors as their experiments showed no effect of gpFI on the DNA nicking activity of terminase *in vitro* (Becker et al., 1988). We have demonstrated that the effect of gpFI depends strongly on the reaction conditions, however, and in particular the enzyme:DNA ratio (Figure 3). Addition of gpFI to the reaction mixture markedly stimulated *in vitro cos*-cleavage under multiple-turnover conditions (excess DNA), but not under single-turnover conditions (excess terminase). Our studies further demonstrate that gpFI-mediated stimulation of *cos*-cleavage results from an increase in *turnover* by the enzyme with little, if any, effect on the *rate* of the reaction. These data suggest that in the absence of viral proheads, gpFI destabilizes complex I, thus allowing terminase release from *cos* and catalytic turnover by the enzyme. On the basis of these data, we favor a model where gpFI acts, at least in part, independent of prohead binding to complex I.

Model for the Initiation of DNA by λ Terminase. On the basis of data available in the literature (Becker & Murialdo, 1990; Feiss, 1986; Feiss & Becker, 1983; Murialdo, 1991; Tomka & Catalano, 1993a,b) and the data presented here, we propose the following model for the initiation of DNA packaging by λ terminase (see Scheme 1). Assembly of the nicking complex initiates with the cooperative binding of gpA, gpNu1, and IHF at *cos* ($T{\cdot}D_{cos}$). ATP, although not strictly required, plays an important role in the assembly process when protein is present in limiting amounts and/or when terminase binding to *cos* is impaired (i.e., by mutation). The endonuclease activity of terminase site-specifically cuts the duplex, yielding the nicked, annealed enzyme•DNA intermediate ($T{\cdot}D_L{\cdot}D_R$). ATP modulates the nicking reaction and ensures that the assembly properly aligns the protein subunits for specific cleavage at N_1 and N_2 (see Figure 1) (Higgins et al., 1988). Strand separation by terminase releases D_R from the enzyme which remains bound to DNA, thus yielding complex I ($T{\cdot}D_L$). An empty prohead next binds to this stable intermediate which decreases the affinity of terminase for *cos*, thus initiating translocation from the mature left end of the genome, and active DNA packaging ensues. We note that the protein composition of each of these packaging intermediates ($T{\cdot}D_{cos}$, $T{\cdot}D_L$, etc.) likely varies and remains speculative at this time (see footnote 2).

We suggest that prohead binding to complex I is necessary, but not always sufficient for terminase release from D_L. We posit that stimulation of packaging by phage λ gpFI protein results from a direct decrease in the binding affinity

Scheme 1: Model for the Initiation of DNA Packaging by λ Terminase[a]



[a] We define initiation of DNA packaging as all of the events from the initial assembly of a nicking complex at *cos* to active DNA packaging into the viral prohead. The terminase subunits (gpA and gpNu1) and IHF cooperatively assemble onto the DNA concatemer at *cos*, forming the multiprotein nicking complex (T•D$_{cos}$). While we show a specific protein composition for the nicking complex (T•D$_{cos}$), the subunit stoichiometry of this intermediate remains speculative at this time (see footnote 2). The endonuclease activity of terminase yields the nicked, annealed duplex (T•D$_L$•D$_R$), and strand separation by the enzyme releases D$_R$ from the complex. An empty viral prohead binds to the binary complex, and prohead•terminase translocation from *cos* ensues, resulting in active DNA packaging by the enzyme. While the mature viral capsid is icosahedral, both the prohead and DNA-filled head are represented as octagons for simplicity. The points where gpFI might stimulate DNA packaging are indicated (dashed arrow, *1 and *2). Details are discussed in the text.

(increased K_d) of terminase for D$_L$. In this model, proheads and gpFI act cooperatively to liberate terminase from *cos* subsequent to DNA nicking and strand separation[4] (*2, Scheme 1). The need for gpFI thus becomes critical when terminase and/or prohead concentrations are limiting. In the absence of proheads, gpFI destabilizes complex I such that terminase "prematurely" dissociates from the cut DNA substrate, thus allowing catalytic turnover by the enzyme (dashed arrow, Scheme 1).[5] The observed rate of nicking remains limited by assembly of the nicking complex (Tomka & Catalano, 1993b), however, and no change in the *rate* of *cos*-cleavage is observed (Figure 3).

Currently accepted models for gpFI-mediated stimulation of DNA packaging suggest that this phage protein promotes complex II formation by increasing the rate of prohead binding to complex I (Becker et al., 1988). Our model suggests that gpFI enhances the rate of DNA packaging by destabilizing complex I, thus making it easier for the prohead•terminase complex to initiate translocation from *cos*. While complex II is known to result from an interaction between proheads and complex I, the exact nature of this intermediate remains obscure. Complex II may in fact represent an intermediate in which packaging has initiated and DNA has at least partially entered the prohead (Becker et al., 1977, 1988). If this is the case, our model predicts that gpFI would indeed stimulate complex II formation, not by directly promoting prohead binding to complex I (*1, Scheme 1), but rather by promoting cooperative release of the prohead•terminase packaging complex from *cos* (*2, Scheme 1). We note here that Becker and co-workers similarly suggested that gpFI might act at a step subsequent to prohead binding to complex I, but their data did not allow them to specify mechanistic details (Becker et al., 1988).

The model presented above predicts that the requirement for gpFI in viral assembly directly depends upon (1) the strength of bonding interactions between terminase and *cos* and (2) the capacity of proheads alone to initiate terminase translocation from *cos*. Two lines of evidence provide further support for our model: (1) Phage 21 is a related lambdoid phage which encodes a terminase enzyme composed of the gene products of *1* (gp1, 181 residues) and *2* (gp2, 308 residues) (Miller & Feiss, 1985). This phage is strongly dependent on IHF for growth (Feiss et al., 1985), and it has been suggested that phage 21 terminase does not bind as tightly as λ terminase to *cos*. Our model predicts that weakened phage 21 terminase•*cos* interactions would attenuate the requirement for gpFI in phage 21 assembly, and consistent with this prediction, phage 21 does not appear to have any dependence on gpFI for growth (Feiss et al., 1988; Smith & Feiss, 1993). (2) Sippy and Feiss have constructed a phage which lacks the last five C-terminal amino acids in gpA (λ A_{am42}) (Sippy & Feiss, 1992), a region known to be important in terminase−prohead bonding interactions (Frackman et al., 1984). Terminase isolated from this mutant phage cuts *cos*-containing DNA efficiently both *in vitro* and *in vivo*, but appears to have a packaging defect consistent with weakened prohead binding interactions (Sippy & Feiss, 1992). Our model predicts that these weakened interactions would yield a phage which is highly dependent on gpFI, and consistent with this prediction, λ A_{am42} phage was significantly more dependent than wild-type on gpFI for growth (Sippy & Feiss, 1992). Finally, we note that second-site suppressors of mutations in the *FI* gene (*fin* for

[4] An alternate mechanism for gpFI action was considered where complex I exists as terminase bound to the nicked annealed duplex (T•D$_L$•D$_R$, Scheme 1). In this model, proheads interact only with T•D$_L$, and packaging is thus limited by the rate of strand separation. gpFI would act by stimulating strand separation and thus appear to stimulate the rate of prohead binding to complex I. In the absence of proheads, T•D$_L$ is unstable, however, and terminase releases from the complex, allowing catalytic turnover by the enzyme (dashed arrow, Scheme 1). While we cannot rigorously exclude this model, most investigators presume that complex I is composed of terminase bound to the strand-separated duplex (T•D$_L$) which favors the model presented in the text. Notwithstanding, we are currently examining the effect of gpFI protein on the strand separation activity of λ terminase.

[5] We note that mature genome left ends may be isolated from *FI*$^+$ phage-infected cells, indicating at least some protection of D$_L$ from nucleases *in vivo*. This suggests that complex I is at least partially stable in the phage-infected cell. It is likely that cooperative interactions between proheads and gpFI are more important within the phage-infected cell and "premature" release of terminase from *cos* is thus prevented *in vivo*.

FI independence) map in the region of the terminase genes (*A* and *Nu1*) (Murialdo et al., 1981a). While it has not been directly demonstrated, we suggest that the *fin* terminase enzymes do not bind *cos*-containing DNA as tightly as wild-type and thus do not require gpFI to package their DNA. Work currently underway in our laboratory is directed at measuring the relative DNA binding affinities of wild-type and *fin* mutant terminase enzymes to test this hypothesis.

It is of interest to compare terminase-mediated DNA packaging with the initiation of transcription by eucaryotic RNA polymerase II (pol II) (Goodrich & Tjian, 1994). Tjian and co-workers have demonstrated that while TATA-binding protein, transcription factors IIB and IIF, and pol II were all necessary and sufficient for the *assembly* of the multiprotein initiation complex, transcription factors IIE and IIH and ATP hydrolysis were required for *promoter clearance*, i.e., movement of pol II away from the open promoter (Goodrich & Tjian, 1994). Interestingly, TFIIH possesses a helicase activity which was proposed to be necessary for movement of pol II from the stable open promoter complex and promoter clearance. Assembly of a terminase nicking complex similarly requires several proteins (gpA, gpNu1, IHF) and ATP (but not ATP hydrolysis). Subsequent to duplex nicking and strand separation, additional proteins (proheads and gpFI) and ATP hydrolysis are necessary to liberate the complex from the assembly site and initiate terminase movement. Similar themes also apply to the initiation of genomic DNA replication by both prokaryotic (Kornberg & Baker, 1992) and viral (Kornberg & Baker, 1992; Mallory et al., 1990) DNA polymerases. These mechanistic similarities suggest that an understanding of the catalytic properties of the terminase packaging complex may yield insight into the general mechanisms of DNA manipulation by large multiprotein enzyme complexes.

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