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Mutations in *Nu1*, the Gene Encoding the Small Subunit of Bacteriophage λ Terminase, Suppress the Postcleavage DNA Packaging Defect of *cosB* Mutations

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The linear double-stranded DNA molecules in λ virions are generated by nicking of concatemeric intracellular DNA by terminase, the λ DNA packaging enzyme. Staggered nicks are introduced at *cosN* to generate the cohesive ends of virion DNA. After nicking, the cohesive ends are separated by terminase; terminase bound to the left end of the DNA to be packaged then binds the empty protein shell, i.e., the prohead, and translocation of DNA into the prohead occurs. *cosB*, a site adjacent to *cosN*, is a terminase binding site. *cosB* facilitates the rate and fidelity of the *cosN* cleavage reaction by serving as an anchoring point for gpNu1, the small subunit of terminase. *cosB* is also crucial for the formation of a stable terminase-DNA complex, called complex I, formed after *cosN* cleavage. The role of complex I is to bind the prohead. Mutations in *cosB* affect both *cosB* functions, causing mild defects in *cosN* cleavage and severe packaging defects. The lethal *cosB* R3$^-$ R2$^-$ R1$^-$ mutation contains a transition mutation in each of the three gpNu1 binding sites of *cosB*. Pseudorevertants of λ *cosB* R3$^-$ R2$^-$ R1$^-$ DNA contain suppressor mutations affecting gpNu1. Results of experiments that show that two such suppressors, *Nu1*ms1 and *Nu1*ms3, do not suppress the mild *cosN* cleavage defect caused by the *cosB* R3$^-$ R2$^-$ R1$^-$ mutation but strongly suppress the DNA packaging defect are presented. It is proposed that the suppressing terminases, unlike the wild-type enzyme, are able to assemble a stable complex I with *cosB* R3$^-$ R2$^-$ R1$^-$ DNA. Observations on the adenosine triphosphatase activities and protease susceptibilities of gpNu1 of the *Nu1*ms1 and *Nu1*ms3 terminases indicate that the conformation of gpNu1 is altered in the suppressing terminases.

Phage λ is one of many DNA viruses that produce concatemeric DNA during lytic development, leading to the requirement that unit-length, virion chromosomes be generated by cleavage of the concatemer. All of the viruses so far studied that process concatemeric DNA also construct a preformed capsid shell, the procapsid or prohead, into which the DNA is translocated; cutting the concatemer accompanies the packaging process. λ virion DNA is a linear duplex 48,502 bp in length, with 12-base, complementary cohesive ends at the 5′ ends of the strands. The cohesive ends allow cyclization of the chromosome upon injection into a cell. Regeneration of the cohesive ends occurs through the introduction, by the phage-encoded enzyme terminase, of nicks staggered by 12 bp at *cosN*, the site containing the cohesive end sequence. *cosN* is just one of three DNA signals in a larger segment called *cos*. *cos* also contains *cosB*, a terminase binding site required for initiation of packaging, and *cosQ*, a site required for termination (Fig. 1).

Terminase consists of a small subunit, gpNu1, the 21-kDa product of the *Nu1* gene, and a large subunit, gpA, the 74-kDa product of the *A* gene (Fig. 1). Terminase is a bivalent DNA binding protein. The endonuclease activity of terminase, which nicks *cosN*, is in gpA (15, 32, 33), and the *cosB* binding activity is in gpNu1 (36). Mutations in gpA that specifically inactivate the endonuclease activity have been identified in the C-terminal third of gpA (15, 26, 27). Binding of *cosB* presumably occurs through a putative helix-turn-helix DNA binding domain at the amino terminus of gpNu1 (5).

Because *cosN* shows partial rotational symmetry, *cosN* is presumably recognized and nicked by symmetrically disposed gpAs (5, 7). There are three gpNu1 binding sites in *cosB*: R3, R2, and R1 (36). Between R3 and R2 is I1, a binding site for the *Escherichia coli* DNA bending protein IHF (28, 42–44). It has been proposed that an IHF-induced bend at I1 facilitates interactions between a terminase bound at R3 with terminases bound at R2 and/or R1 (11, 28, 42, 44). The nonspecific DNA bending protein HU also assists in the terminase-*cosB* interaction (30). The terminase-*cosB* interaction stimulates the efficiency and fidelity of *cosN* cleavage, presumably by anchoring and positioning terminase for proper nicking of *cosN* (13, 22–24). Terminase is an adenosine triphosphatase (ATPase); gpNu1 contains a DNA-stimulated, low-affinity ATPase activity (K_m, ≈500 μM), and gpA contains a high-affinity ATPase activity (K_m, ≈5 μM) (20, 27, 40). The nicking activity of terminase is stimulated by the presence of either ATP or a poorly hydrolyzed ATP analog, indicating that ATP binding, but not hydrolysis, is required for stimulation of endonuclease activity. Following *cosN* nicking, the newly created cohesive ends are separated by terminase in a reaction requiring ATP hydrolysis, followed by the formation of complex I, a stable association of terminase with the left end of the DNA molecule to be packaged; this association involves interactions between terminase and *cosB* (13). Complex I binds a prohead to form complex II, and translocation of DNA into the prohead ensues. Terminase remains part of the translocating complex and is available to cleave the downstream *cos* to terminate packaging (18, 19). Terminase is speculated to play a role in translocation (5, 7, 16). *cosQ* is required for proper termination (12).

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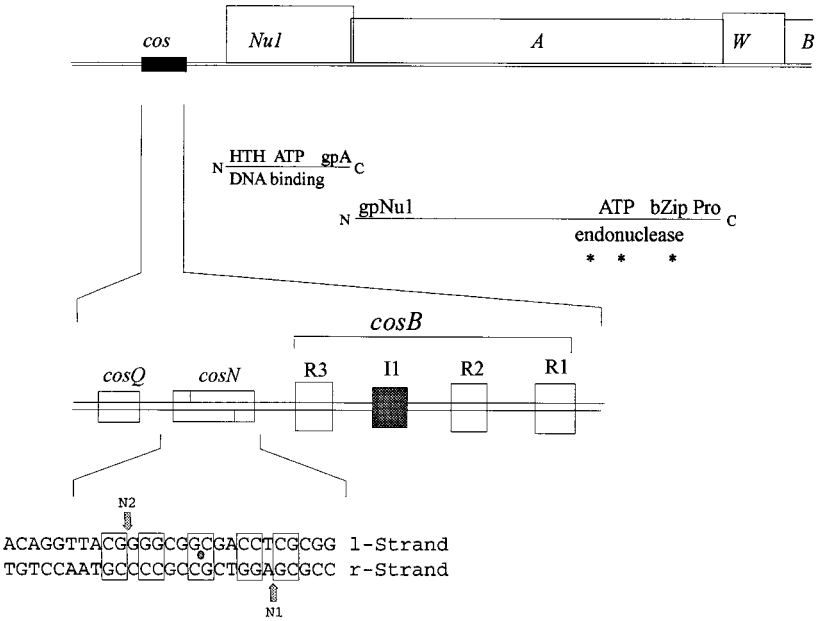

FIG. 1. *cos* and the terminase genes. The *Nu1* and *A* genes encode the small (gpNu1) and large (gpA) subunits of terminase, and gene *B* encodes the portal protein of the prohead. Below the *Nu1* and *A* genes are diagrams of the gpNu1 and gpA polypeptides and their domain structures (for reviews, see references 5, 7, and 17). For gpNu1, HTH denotes the location of a putative helix-turn-helix DNA binding motif, ATP indicates a match to known ATPases (3) and the likely site of gpNu1 ATPase activity, and gpA indicates the domain for binding gpA. For gpA, gpNu1 and Pro indicate the sites of interaction with gpNu1 and the portal protein of the prohead, respectively; ATP indicates a putative ATPase center and the likely site of gpA ATPase activity, and bZip indicates a putative basic leucine zipper (15). N and C termini are marked. Asterisks indicate sites where mutational changes specifically abolish the endonuclease activity of terminase. *cos* is tripartite, consisting of *cosQ* (the termination signal), *cosN* (the nicking site), and *cosB* (the terminase binding site). The three R sequences of *cosB* are sites bound by gpNu1, presumably through the helix-turn-helix, and I1 is a binding site for the integration host factor, IHF, the DNA binding and bending protein of *E. coli*. The exact extents of sites are not known; the base pair coordinates for the conserved R site sequences are bp 53 to 64 for R3, bp 109 to 120 for R2, and bp 155 to 166 for R1. At the bottom of the figure, the segment of twofold rotational symmetry of *cosN* is shown; the base pairs showing rotational symmetry are boxed, and the center of symmetry is indicated by a dot. The normal nick sites, N1 and N2, are indicated. l-Strand, left strand; r-Strand, right strand.

Mutations in *cosB* appear to affect *cos* cleavage and a post-cleavage step of DNA packaging, as follows. In a genetic study of *cosB* (10), an R sequence mutation (2) known to affect gpNu1 binding (36), was introduced into each of the R sequences to generate the *cosB* R3$^-$ R2$^-$ R1$^-$ triple mutation. In the presence of 1 mM ATP, the *cosB* R3$^-$ R2$^-$ R1$^-$ mutation had a 3-fold effect on in vitro *cos* cleavage (13) but in vivo was found to prevent plaque formation and to cause a 1,000-fold defect in virion production (10). These results led to the proposal that *cosB* plays a role in DNA packaging in addition to its role in *cos* cleavage. Accordingly, suppressors of *cosB* defects might suppress either or both *cosB* defects. Suppressors of the *cosB* R3$^-$ R2$^-$ R1$^-$ mutation have been isolated and found to be missense mutations in *Nu1*. Two such suppressors were *Nu1*ms1 and *Nu1*ms3, which caused the amino acid changes $L_{40}F$ and $Q_{97}K$ in gpNu1, respectively (11). To understand how these suppressor mutations act at the molecular level, we have compared the gpNu1ms1 and gpNu1ms3 terminases with the wild-type enzyme.

MATERIALS AND METHODS

Sequence designations. The numbering convention of Daniels et al. (14) is used. Numbering of the λ sequence begins with the first base of the left cohesive end and continues along the left strand (the top strand) in a 5′-to-3′ direction. The positions of restriction enzyme cleavage sites are given as the first nucleotides of the recognition sequences.

Media, bacteria, phages, and plasmids. Luria broth (1) was the standard medium; it was supplemented with ampicillin (100 μg/ml) when required. The terminase expression strain, OR1265(pCM101), was obtained from H. Murialdo (8). The prohead source, MF2517, was a derivative of MF1427, a *galK* mutant of the *E. coli* C strain C1a (38); MF2517 carries the prophage λ *c*I857 *S*am7 Δ(*cos-Nu1-A*)::Kn^r (25). For *cos* cleavage assays, the *cos*$^+$ substrate was pSX1, a derivative of pUC19 (41) carrying a λ insert extending from the *Bcl*I site at bp 47942 through *cos*$^+$ to an *Eco*RI site at bp 194. An analogous plasmid carrying the R3$^-$ R2$^-$ R1$^-$ point mutations of *cosB* was described by Cue and Feiss (10); the vector for this plasmid was pIBI31 (International Biotechnologies, Inc.). A third plasmid, with *cosB* deleted, was prepared by oligonucleotide-directed mutagenesis of an M13mp19 derivative, which carries the *Bcl*I-to-*Eco*RI insert of pSX1 (45). The mutagenesis changed bp 54 from GC to CG and created a *Stu*I site at bp 52. Following mutagenesis, the *Bcl*I-to-*Eco*RI insert was transferred from M13mp19 replicative-form DNA into pUC19 to generate pZHC1. Plasmid pZHC1 was successively treated with *Eco*RI, DNA polymerase (Klenow fragment) in the presence of the four deoxyribonucleotides to fill in the cohesive ends, *Stu*I, and T4 DNA ligase, resulting in pZHC2, which carries a Δ*cosB* mutation, i.e., a deletion of base pairs between the *Stu*I and *Eco*RI sites, i.e., Δ(53-194). For DNA packaging, *cos*$^+$ DNA was commercial λ *c*I857 *S*am7 DNA; λ *cosB* R3$^-$ R2$^-$ R1$^-$ DNA was from CsCl-purified virions of λ P1:5R *nin*5 Kn^r *c*I857 *cosB* R3$^-$ R2$^-$ R1$^-$ *Nu1*ms1 (11).

PCR amplifications. For PCR amplifications of *cos* segments for competition experiments, *cos*-containing inserts were transferred to the vector pIBI31. The primers used were (i) a primer that annealed to λ bp 48421 to 48437 and (ii) a primer that annealed to the T3 promoter of pIBI31 adjacent to the *Eco*RI site used for insertion of the λ segment. These primers result in amplification of a 305-bp *cos*-containing DNA segment; standard PCR techniques were used (9, 34).

Terminase and proheads. Terminase was overexpressed by heat induction of OR1265 cells carrying the terminase expression vector pCM101 (8). Plasmid pCM101 produces wild-type terminase, and derivatives of pCM101 expressing *Nu1*ms1 and *Nu1*ms3 terminases were constructed by standard recombinant DNA techniques (35, 37). Terminase was purified by a modification (25) of the method of Tomka and Catalano (39). Proheads were supplied as a sonic extract of induced MF2517 cells.

ATPase assays. ATPase assays were carried out and analyzed as described previously (27).

***cos* cleavage in vitro.** Reactions were carried out as described by Chow et al. (8). The DNA substrate was the 3.3-kb cosmid pSX1 (45), linearized by digestion with *Sca*I. Cleavage of linearized pSX1 by terminase generates 1.0- and 2.3-kb products. The standard reaction mixture contained 9 nM substrate DNA and 50 nM purified terminase, assuming two gpNu1 subunits and one gpA subunit per terminase (39). The reaction was stopped at 10 min by addition of EDTA (pH 8.0) to 25 mM. Samples were heated to 65°C for 10 min and subjected to 0.8%

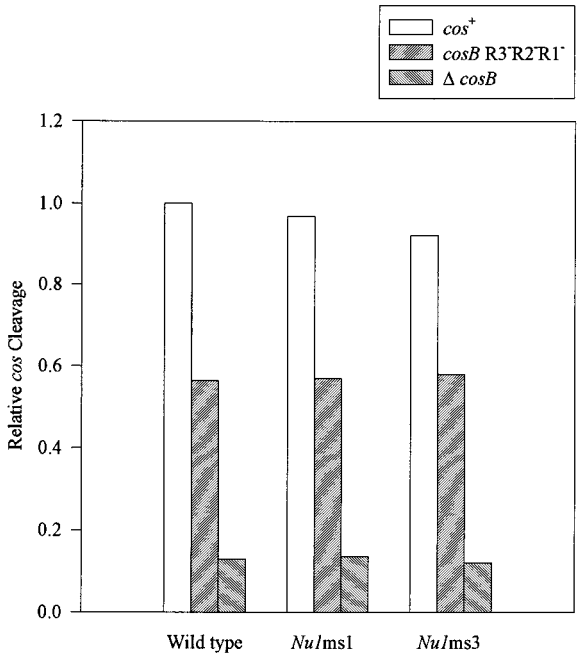

FIG. 2. *cos* cleavage by wild-type, *Nu1*ms1, and *Nu1*ms3 terminases. Linearized cosmid DNAs containing cos^+, *cosB* $R3^-$ $R2^-$ $R1^-$, or Δ*cosB* DNA sites were used as substrates in *cos* cleavage reactions. The extent of *cos* cleavage is normalized to that for cos^+ DNA; the extent of cleavage of the cos^+ DNA was 17.5%.

agarose gel electrophoresis. The DNA was transferred to a GeneScreen membrane and probed with ^{32}P-labelled pSX1 DNA, and the extent of cleavage was determined by scanning in a phosphorimager apparatus. Control experiments showed that the *cos* cleavage assay was linear with respect to terminase concentration up to 125 nM and linear with respect to time for 20 min.

In vitro DNA packaging. The 60-μl reaction mixture in DPB buffer (8) contained 1 mM ATP, 1.7×10^{-2} pmol of λ DNA (0.5 μg), 0.5 pmol of terminase (60 ng), and 20 μl of prohead crude extract. Incubation at room temperature (22°C) was stopped at 20 min by addition of DNase I to 10 μg/ml, and the mixtures were incubated for 20 min at room temperature. RNase I was added to 20 μg/ml, EDTA (pH 8.0) was added to 25 mM, and the samples were incubated for 20 min at room temperature. Then sodium dodecyl sulfate (SDS) to 1% and proteinase K to 500 μg/ml were added and the reaction mixtures were incubated at 55°C for 30 min. The volume was reduced by vacuum centrifugation, loading buffer was added, and the samples were subjected to electrophoresis in 0.8% agarose. Control experiments showed that the DNA packaging assay was linear with respect to terminase concentrations of up to 100 ng/reaction mixture and linear with respect to time for 40 min.

Protease digestions. For trypsin digestions of terminase, the standard 15-μl reaction mixture contained 10 mM Tris-HCl (pH 8.0), 0.01% Tween 20, 10 mM $CaCl_2$, and various concentrations of trypsin. Mg-ATP and λ DNA, when added, were at 1 mM and 3×10^{-4} μM (11 μg/ml), respectively. Incubation was for 60 min at 37°C. Samples were subjected to SDS-polyacrylamide gel electrophoresis on a 12.5% gel, and peptides were detected by silver staining (Bio-Rad, Inc.). In some reaction mixtures, Chromozym TRY, a chromogenic trypsin substrate (Boehringer Mannheim, Inc.), was used as an internal control; this control showed that the trypsin hydrolysis rate was the same in all reactions.

RESULTS

The *cos* cleavage activities of the *Nu1*ms1 and *Nu1*ms3 terminases. To determine whether the *Nu1*ms1 and *Nu1*ms3 mutations alter the *cos* cleavage ability of terminase, linearized cosmid DNA was digested with terminase and the extent of *cos* cleavage was determined as described in Materials and Methods. Purified wild-type *Nu1*ms1 and *Nu1*ms3 terminases were used in *cos* cleavage experiments with cos^+, *cosB* $R3^-$ $R2^-$ $R1^-$, and Δ*cosB* cosmid DNAs. With wild-type enzyme, the *cosB* $R3^-$ $R2^-$ $R1^-$ DNA was cut about half as efficiently as cos^+ DNA and Δ*cosB* DNA was cut with an efficiency that was reduced approximately 10-fold (Fig. 2). The cleavage efficiencies of the *Nu1*ms1 and *Nu1*ms3 terminases were not significantly different from that of wild-type enzyme. The *cos* cleavage result was confirmed by a competition experiment in which short, PCR-generated *cos* segments (cos^+ or *cosB* $R3^-$ $R2^-$ $R1^-$) were used as competitors in the *cos* cleavage reaction (Fig. 3). The *cosB* $R3^-$ $R2^-$ $R1^-$ DNA was a poor competitor for all three enzymes, a result not expected if the *Nu1*ms terminases could bind the *cosB* $R3^-$ $R2^-$ $R1^-$ DNA better than the wild-type enzyme. The results show that the mutant terminases do not differ greatly in *cos* cleavage activity and indicate that the *Nu1*ms1 and *Nu1*ms3 mutations do not improve the ability of wild-type enzyme to bind or cut the *cosB* $R3^-$ $R2^-$ $R1^-$ DNA, under the conditions used. The results indicate that the *Nu1*ms terminases do not differ significantly from wild-type terminase in assembly of a prenicking complex and *cosN* nicking.

The packaging defect of *cosB* mutations: suppression by the *Nu1*ms1 and *Nu1*ms3 terminases. We examined the effects of the *Nu1*ms1 and *Nu1*ms3 mutations on post-*cos* cleavage steps of DNA packaging by looking at DNA packaging using a DNase I protection assay. In this assay, packaging mixtures containing terminase, ATP, and proheads were used to package virion DNAs, i.e., virus DNAs containing cohesive ends. After the packaging reactions, unpackaged DNAs were digested with DNase I and the packaged DNAs were isolated, subjected to agarose gel electrophoresis, and quantitated by Southern hybridization. In reactions with wild-type terminase, cos^+ DNA was packaged about 12-fold more efficiently than *cosB* $R3^-$ $R2^-$ $R1^-$ DNA, indicating that the *cosB* $R3^-$ $R2^-$ $R1^-$ mutations cause a substantial defect in packaging (Fig. 4). In contrast, the *Nu1*ms1 and *Nu1*ms3 terminases packaged the *cosB* $R3^-$ $R2^-$ $R1^-$ DNA about as efficiently as the cos^+ DNA, indicating that the mutant terminases are significantly better at packaging the *cosB* $R3^-$ $R2^-$ $R1^-$ DNA than is the wild-type enzyme. We conclude that the *Nu1*ms1 and *Nu1*ms3 mutations affect the efficiency of packaging of λ *cosB* $R3^-$ $R2^-$

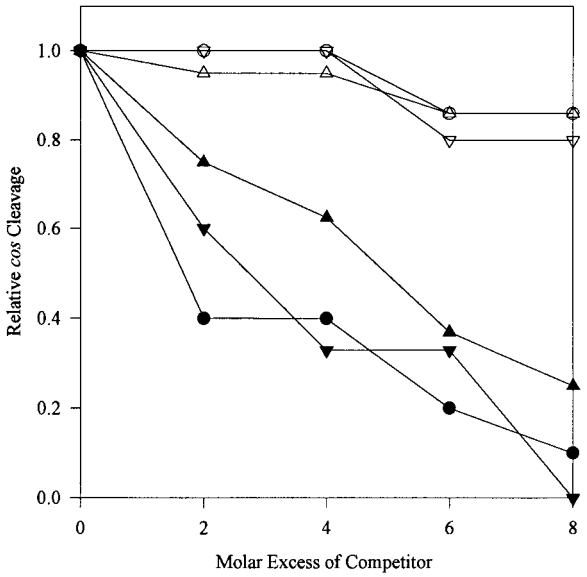

FIG. 3. Cleavage of cos^+ DNA by wild-type (circles), *Nu1*ms1 (triangles), and *Nu1*ms3 (inverted triangles) mutant terminases: the effect of competitor DNA. Competitor DNAs, namely, cos^+ (closed symbols) and *cosB* $R3^-$ $R2^-$ $R1^-$ (open symbols) DNAs, were generated by PCRs. The *cos* cleavage reaction was done as described in Materials and Methods.

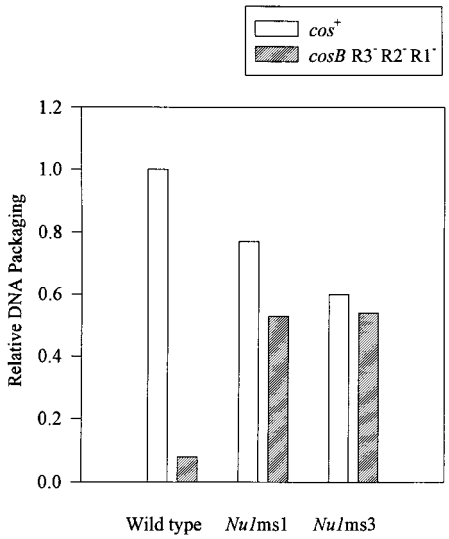

FIG. 4. DNA packaging by wild-type, *Nu1*ms1, and *Nu1*ms3 terminases. λ *cos*$^+$ or λ *cosB* R3$^-$ R2$^-$ R1$^-$ DNAs were used as packaging substrates in packaging reactions with purified terminase and crude proheads. A DNase I protection assay of packaged DNA was used.

R1$^-$ DNA and that the mutations affect a packaging step occurring after *cos* cleavage. Note that the 2-fold defect in *cos* cleavage and the 12-fold defect in packaging of λ *cosB* R3$^-$ R2$^-$ R1$^-$ DNA by wild-type terminase observed in vitro do not account for the 1,000-fold defect in virion production found in vivo; this point is taken up in the Discussion. Also, the *Nu1*ms1 and *Nu1*ms3 terminases packaged *cos*$^+$ DNA somewhat less efficiently than the wild-type enzyme, results that agree with in vivo observations that λ *cos*$^+$ *Nu1*ms1 and λ *cos*$^+$ *Nu1*ms3 produce smaller yields than λ *cos*$^+$ (11).

The ATPase activities of the *Nu1*ms1 and *Nu1*ms3 terminases. Wild-type terminase has two ATPase activities, a low-affinity (K_m, ≈500 μM) center in gpNu1 and a high-affinity (K_m, ≈5 μM) center in gpA (26, 40). The kinetic constants for ATP hydrolysis for the mutant terminases differed mildly from those of wild-type terminase in that the low-affinity ATPase center of the *Nu1* subunit of each of the two mutant terminases showed an affinity for ATP that was about threefold higher than that of the wild-type enzyme; the other constants were unchanged (Table 1).

Conformational studies of *Nu1*ms1 and *Nu1*ms3 terminases. Terminase is involved in a series of nucleoprotein assemblies in proceeding from the prenicking complex through the translocation complex (see Fig. 6). It is likely that the transitions from one complex to the next involve conformational changes in terminase. In addition, the observation that ATP enhances the accuracy and efficiency of the nicking reaction indicates that ATP modifies terminase conformation in the prenicking complex (22–24). The first known DNA packaging intermediate following *cos* cleavage is complex I, a tight association of terminase with the left (*cosB*-containing) chromosome end (7, 13). We considered the possibility that wild-type terminase was unable to efficiently form complex I when the DNA contained the *cosB* R3$^-$ R2$^-$ R1$^-$ mutations because of an inability to undergo a required conformational change. This notion suggested that the *Nu1*ms1 and *Nu1*ms3 mutations might confer either a required conformation or the ability to efficiently make a conformational change with *cosB* R3$^-$ R2$^-$ R1$^-$ DNA. Accordingly, we examined the protease susceptibilities of wild-type and mutant terminases in the presence and absence of ATP and DNA ligands.

TABLE 1. Kinetic parameters of terminases

Terminase	Low-affinity ATP binding site (gpNu1)		High-affinity ATP binding site (gpA)	
	k_{cat}[a] (min^{-1})	K_m (μM)	k_{cat} (min^{-1})	K_m (μM)
Wild type	92	670	40	5.5
*Nu1*ms1	106	245	34	4.3
*Nu1*ms3	84	203	34	4.4

[a] k_{cat}, catalytic constant.

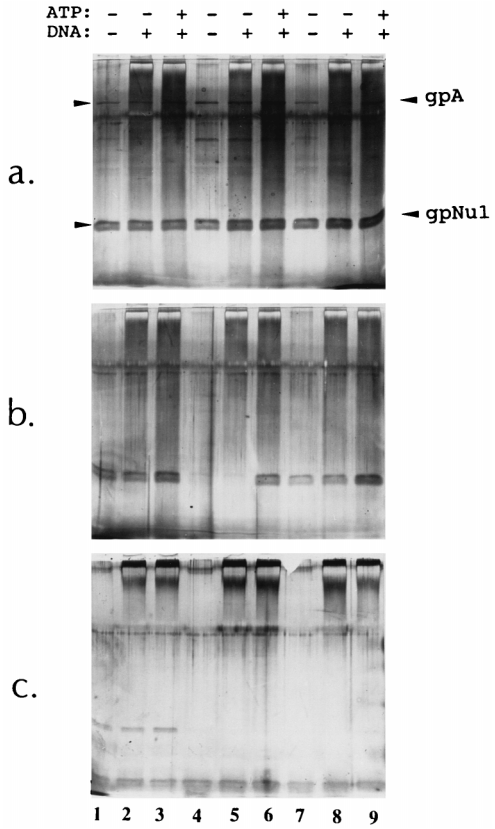

FIG. 5. Trypsin digestion of wild-type, *Nu1*ms1, and *Nu1*ms3 terminases: effects of ATP and DNA ligands. Lanes 1 to 3, *Nu1*ms1 terminase; lanes 3 to 5, *Nu1*ms3 terminase; lanes 7 to 9, wild-type terminase. The amounts of trypsin added were 3.3, 10, and 26.4 ng for reactions in panels a, b, and c, respectively. The reaction was for 60 min at 37°C. The SDS-polyacrylamide gel was developed with silver stain to detect polypeptides.

The trypsin susceptibilities of wild-type, *Nu1*ms1, and *Nu1*ms3 terminases were determined by digestions at three trypsin concentrations (Fig. 5). At the intermediate trypsin concentration, digestion of the gpNu1 subunit of the *Nu1*ms3 terminase was apparent (Fig. 5b, lanes 4 to 6). At the highest trypsin concentration, cleavage of the *Nu1* subunits of the *Nu1*ms3 and wild-type terminases was complete, whereas a considerable fraction of the gpNu1 subunit of the *Nu1*ms1 terminase was intact (Fig. 5c). Similar stability results were obtained when chymotrypsin and V8 protease were used (not shown). Comparison of a number of digestions indicated that the *Nu1*ms1 and *Nu1*ms3 mutations have opposite effects on the protease susceptibility of the gpNu1 subunit of holoterminase. The gpNu1 subunit of *Nu1*ms3 terminase is two- to threefold more susceptible to

protease digestion than wild-type terminase, and the *Nu1*ms1 gpNu1 subunit is about eightfold more resistant to protease digestion than the wild-type enzyme. Effects of the *Nu1*ms1 and *Nu1*ms3 mutations on the stability of the gpA subunit were not obvious. In addition to the effects of the *Nu1*ms mutations on gpNu1 stability to proteases, effects of ATP and DNA ligands were noted, as follows. ATP addition resulted in enhanced stability of the gpA subunits of both wild-type and mutant terminases, and this enhancement did not require ATP hydrolysis, as the poorly hydrolyzed analog ATP-γ-S also stabilized gpA (data not shown). Addition of both ATP and DNA enhanced the stability of both the gpNu1 and gpA subunits of both the wild-type and mutant enzymes for trypsin, chymotrypsin, and V8 protease (Fig. 5 and data not shown).

DISCUSSION

The *Nu1*ms1 and *Nu1*ms3 mutations are general suppressors of *cosB*-associated DNA packaging defects (11, 21, 31, 42, 44). Although the *Nu1*ms suppressors are able to suppress a variety of *cosB* mutations, the suppressors do not act by totally bypassing the *cosB* requirement; the *Nu1*ms suppressors are unable to promote the packaging of cosmids from which all of *cosB* is deleted, indicating that the suppressors act through increased efficiency of interaction with *cosB* by the suppressing terminases (11).

cosB and ATP act in concert to stimulate the nicking of *cosN* by terminase. The absence of *cosB* or ATP results in nicking that is less accurate and less efficient (13, 22–24). These observations suggest that *cosB* serves as an anchoring site for terminase and that the binding of ATP alters the conformation of terminase to properly position gpA for efficient and accurate *cosN* nicking. Although *cosB* R3$^-$ R2$^-$ R1$^-$ is cut with only a modest 2-fold reduction in efficiency (Fig. 2), virion production in vivo by λ *cosB* R3$^-$ R2$^-$ R1$^-$ DNA is reduced 1,000-fold from the λ *cos*$^+$ level (10). The severe defect in virion production led to the proposal that λ *cosB* R3$^-$ R2$^-$ R1$^-$ has a post-*cos* cleavage defect in addition to the mild *cos* cleavage defect (10). Here we find that mature λ *cosB* R3$^-$ R2$^-$ R1$^-$ DNA is packaged 12-fold less well than λ *cos*$^+$ DNA, a defect considerably more severe than the *cos*-cleavage defect (Fig. 4). The packaging defect found for λ *cosB* R3$^-$ R2$^-$ R1$^-$ DNA provides support for the previous proposal of a postcleavage role for *cosB*. Although we find that the post-*cos* cleavage defect of λ *cosB* R3$^-$ R2$^-$ R1$^-$ DNA is more severe than the *cos* cleavage defect, the combination of the two defects leads only to about a 25-fold defect overall, which is much less than the 1,000-fold defect found for λ *cosB* R3$^-$ R2$^-$ R1$^-$ in vivo. There are several possible explanations for the difference in severity between the in vitro and in vivo defects caused by *cosB* R3$^-$ R2$^-$ R1$^-$. First, the DNase protection assay for in vitro DNA packaging has a background level of nonspecific DNA packaging, i.e., packaging of non-λ linear DNA (12), and so the packaging observed for *cosB* R3$^-$ R2$^-$ R1$^-$ DNA likely includes DNA packaged via the normal packaging pathway plus DNA packaged via a nonspecific mechanism. The nonspecific mechanism is expected to package some DNA molecules with a polarity opposite to the normal, *cosB*-first orientation. Second, it is possible that there is an additional packaging defect caused by *cosB* R3$^-$ R2$^-$ R1$^-$; one possibility is that *cosB* R3$^-$ R2$^-$ R1$^-$ causes a defect in the strand separation step that occurs after nicking and before packaging. Finally, the in vitro assay conditions may not reflect in vivo conditions; the terminase concentration relative to the λ DNA concentration may be lower in vivo than in vitro and hence account for the severity of the *cosB* R3$^-$ R2$^-$ R1$^-$ defects in vivo.

To determine how the *Nu1*ms1 and *Nu1*ms3 mutations suppress *cosB* R3$^-$ R2$^-$ R1$^-$, we examined wild-type, *Nu1*ms1, and *Nu1*ms3 terminases for *cosN* cleavage and DNA packaging of *cos*$^+$ and *cosB* R3$^-$ R2$^-$ R1$^-$ DNAs. In the *cosN* cleavage assays, *cosB* R3$^-$ R2$^-$ R1$^-$ DNA is cut about half as efficiently as *cos*$^+$ DNA and the Δ*cosB* DNA has a 10-fold defect, results consistent with those of previous studies (13). Under the conditions used here, *cosB* clearly stimulates the *cos* cleavage reaction. In the packaging scheme in Fig. 6, mutations in *cosB* are proposed to destabilize the prenicking complex by increasing k_{off1}, the rate constant of terminase dissociation. The *Nu1*ms1 and *Nu1*ms3 mutations do not alter the *cosN* cleavage activity of terminase with any of the substrates used. Because cleavage is clearly stimulated by the presence of *cosB*, the fact that the mutant terminases are unaltered in the *cosN* cleavage reaction indicates that the interaction between *cosB* and terminase involved in *cosN* cutting is unaffected by the *Nu1*ms1 and *Nu1*ms3 mutations, i.e., k_{off1} is not altered in the *Nu1*ms1 and *Nu1*ms3 mutant terminases. In contrast to the results of the *cosN* cleavage assays, *cosB* R3$^-$ R2$^-$ R1$^-$ caused a stronger defect in packaging by wild-type terminase and the *Nu1*ms1 and *Nu1*ms3 terminases restored the efficiency of packaging to near the wild-type level. These contrasting results suggest that terminase-*cosB* interactions are more critical for postcleavage packaging steps than for the nicking reaction or that there are differences between how *cosB* is utilized for *cosN* cleavage and for assembly or stability of complex I. Since the major defect caused by the *cosB* mutations studied to date is post-*cos* cleavage, it makes sense that the suppressors act to correct the post-*cos* cleavage defect rather than to correct the minor defect in *cos* cleavage.

Following the introduction of nicks and the separation of the cohesive ends by terminase, the next known DNA packaging intermediate is complex I, a stable association of terminase with the left end of the chromosome to be packaged (Fig. 6). Complex I has been identified as a stable complex that can be separated from soluble terminase on sucrose gradients; fractions containing complex I generated infectious virions when added to proheads, tails, and the tail attachment proteins gpW and gpFII (4, 6). Complex I formation also accounts for the single-turnover kinetics observed in the in vitro *cos* cleavage reaction (39). Complex I has also apparently been observed in in vivo studies of the fate of λ DNA that has been cut at *cos* but not packaged. In these studies, it was found that newly created right chromosomal ends are unstable, presumably because of attack by intracellular nucleases such as RecBCD. In contrast, the newly created left, or *cosB*-containing, chromosomal ends are stable, and it is proposed that the protection is due to a stable association of terminase with the left end; this terminase-left end complex blocks exonuclease action (29, 37). Whether the complex I identified in vitro is identical to the stable in vivo complex awaits biochemical analysis of each, but because each is a stable, postcleavage complex, it is a reasonable assumption that they are the same complex. Complex I binds a prohead to form complex II, leading to DNA translocation and tail attachment for phage formation (Fig. 6).

The assembly pathway is interrupted when the λ DNA contains a defect in *cosB*. Although in vitro *cos* cleavage studies predict that λ *cosB* R3$^-$ R2$^-$ R1$^-$ should have only a mild defect in *cos* cleavage in vivo, no evidence of *cos* cleavage is found when intracellular DNA is examined. A speculative explanation for this discrepancy is that there actually is significant *cos* cleavage during a λ *cosB* R3$^-$ R2$^-$ R1$^-$ infection but that weakened *cosB*-gpNu1 interactions lead either to (i) rapid dissociation of terminase from the nicked complex, i.e., increased k_{off2} (Fig. 6), or (ii) rapid disassembly of complex I and

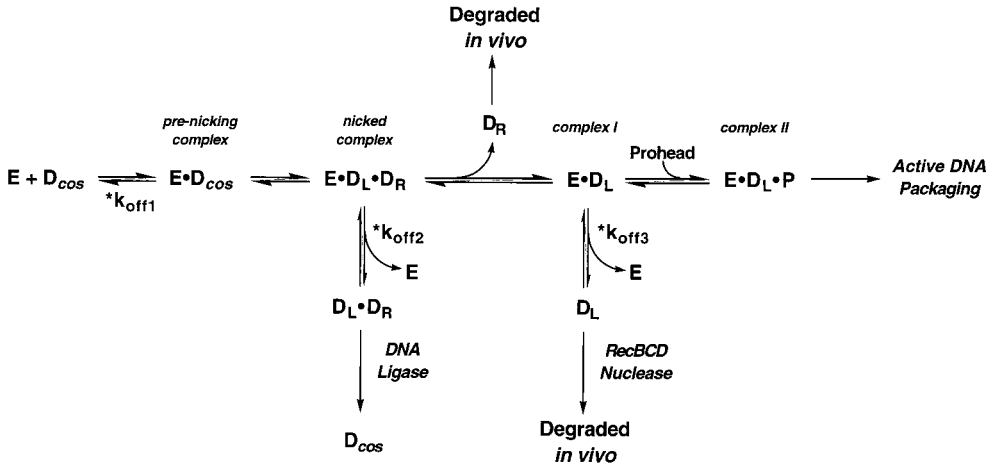

FIG. 6. Model for DNA packaging by phage λ and the effects of *cosB* mutations. Chromosome packaging begins with the assembly of terminase holoenzyme (E) at the *cos* site of λ DNA (D_{cos}), forming a binary enzyme-DNA prenicking complex ($E\bullet D_{cos}$). Site-specific nicking at *cosN* yields the nicked complex, i.e., the nicked, annealed *cos* bound by terminase ($E\bullet D_L\bullet D_R$, where D_L and D_R are the left and right ends of the λ chromosome). Enzyme-mediated strand separation generates complex I, which is terminase bound to the mature left end of the λ chromosome ($E\bullet D_L$). Strand separation also releases the right chromosomal end, which is subject to exonuclease degradation in vivo. Next, complex I binds a prohead, forming complex II, a ternary enzyme-DNA-prohead (P) complex ($E\bullet D_L\bullet P$), and translocation of the DNA into the prohead ensues. Normally k_{off2} and k_{off3}, the rate constants for dissociation of the enzyme-bound nicked duplex and for dissociation of complex I, respectively, are low relative to those for the productive packaging pathway. The *cosB* R3$^-$ R2$^-$ R1$^-$ mutation is proposed to increase k_{off2}, resulting in premature disassembly of the nicked complex; religation of the nicked, annealed DNA duplex ($D_R\bullet D_L$) yields a resealed *cos*. *The cosB* R3$^-$ R2$^-$ R1$^-$ mutation may also increase k_{off3} such that terminase dissociates from complex I prior to exonuclease damage to the right chromosomal end, followed by efficient reannealing and religation of the cohesive ends. The *cosB* $\Delta R_{2,1}$ mutation is proposed to result in an unstable complex I, i.e., k_{off3} is increased. For λ *cosB* $\Delta R_{2,1}$, formation of complex I releases the right chromosomal end (D_R), which is subject to exonuclease attack. Later, dissociation of terminase from the *cosB* $\Delta R_{2,1}$ complex I results in release of the left chromosomal end, which is also subject to exonuclease attack. Not depicted are two host proteins, IHF and HU, which assist terminase binding to *cosB*, and gpFI, which assists prohead binding by complex I. The protein compositions of the packaging intermediates are not known. See Discussion for details and proposals for suppression of *cosB* defects by the *Nu1*ms1 and *Nu1*ms3 mutations.

efficient reannealing of the cohesive ends, i.e., increased k_{off3} (Fig. 6) followed by efficient and rapid ligation of the nicks (13). *cos* resealing would account for the absence of detectable *cosN* cleavage during λ *cosB* R3$^-$ R2$^-$ R1$^-$ infection. It is interesting to contrast the in vivo DNA processing defect of λ *cosB* R3$^-$ R2$^-$ R1$^-$ with that of λ *cosB* $\Delta R_{2,1}$, a milder *cosB* mutation (13). The *cosB* $\Delta R_{2,1}$ mutation is a deletion of R2 and R1 of *cosB*, and the mutation reduces the virus yield to 2.6 phage/cell (versus about 90 phage/cell for λ *cos*$^+$ DNA), a level sufficiently low to prevent plaque formation (10). In contrast to λ *cosB* R3$^-$ R2$^-$ R1$^-$ DNA, the intracellular λ DNA with the *cosB* $\Delta R_{2,1}$ mutation shows efficient nicking at *cosN* (13). The left chromosomal end restriction fragment of λ *cosB* $\Delta R_{2,1}$ DNA has a broad size distribution, indicating that extensive degradation of the left-end DNA occurs following *cosN* cleavage, whereas the left chromosomal end of the *cosB*$^+$ phage is stable. We interpret this result to indicate that wild-type terminase forms a complex I with *cosB* $\Delta R_{2,1}$ that is less stable than the terminase-*cosB*$^+$ association, i.e., k_{off3} is increased (Fig. 6). The complex I of λ *cosB* $\Delta R_{2,1}$ DNA is proposed to be stable enough that degradation of the right chromosomal end occurs prior to terminase dissociation, so that when the left chromosomal end is released by terminase, the right end to which it might anneal has already been attacked by intracellular nucleases. Subsequent dissociation of terminase from complex I leaves left ends vulnerable to attack by intracellular exonucleases. The *Nu1*ms1 and *Nu1*ms3 suppressors allow plaque formation by λ *cosB* $\Delta R_{2,1}$ (11), increasing the yield to about half that of λ *cos*$^+$ (26a). Furthermore, the *Nu1*ms1 and *Nu1*ms3 suppressors result in stabilization of the chromosomal left end (26a), indicating that the suppressor terminases are able to form a stable complex I following cleavage of *cosB* $\Delta R_{2,1}$ DNA (i.e., k_{off3} is decreased by the *Nu1*ms1 and *Nu1*ms3 mutations).

An interesting issue is why *cosB* plays a major role in DNA packaging and a minor role in *cos* cleavage. For *cos* cleavage, terminase interacts with two duplex sites, *cosN* and *cosB*, in a prenicking complex; in complex I, the DNA partners in the complex consist of duplex *cosB* and the single-stranded left cohesive end (Fig. 6). We speculate that, in the conversion of the prenicking complex to complex I, a structural transition occurs such that new contacts are made between terminase and *cosB*. It is also likely that contacts between the left cohesive end and terminase are also important in complex I.

Observations on the effects of the *Nu1*ms1 and *Nu1*ms3 mutations on ATP hydrolysis and conformation may yield clues as to how the suppressors act at the molecular level. The mutant terminases show a mildly enhanced affinity, i.e., two- to threefold, for ATP at the low-affinity site of gpNu1 (Table 1). There is evidence for an interaction between the low-affinity ATPase center of gpNu1 and DNA binding by terminase, as follows. The proposed ATPase and helix-turn-helix domains of gpNu1 are adjacent, and gpNu1 ATPase activity is stimulated by the presence of nonspecific DNA (39). In addition, mutations changing the putative ATPase center of gpNu1 decrease both the specific and nonspecific DNA binding ability of terminase (26a, 27). Thus, the alteration of the affinity of the gpNu1 ATPase center for ATP, by *Nu1*ms1 and *Nu1*ms3, may reflect this interaction between the ATPase center and the DNA binding domain of gpNu1.

The suppressor mutations cause substantial changes in the susceptibility of gpNu1 to protease digestion, with *Nu1*ms1 terminase having about an eightfold-decreased susceptibility to proteases and *Nu1*ms3 terminase having a two- to threefold-increased susceptibility. Clearly, the *Nu1*ms1 and *Nu1*ms3 mutations have different effects on the structure of gpNu1 in holoterminase. Given that terminase likely undergoes a conformational transition during formation of complex I, the

changes caused by the *Nu1*ms1 and *Nu1*ms3 mutations may facilitate the transition that is presumably mediated by the binding of *cosB*. Because the in vivo terminase-*cosB* complex of λ *cosB* $\Delta R_{2,1}$ is less stable than the complex of wild-type terminase with λ *cos*$^+$ DNA, and because the *Nu1*ms mutations suppress both the lethality and the post-*cos* cleavage degradation of intracellular DNA caused by *cosB* $\Delta R_{2,1}$, we speculate that the *Nu1*ms mutations result in a more stable association of terminase with the truncated *cosB* $\Delta R_{2,1}$ DNA. Thus, we propose that the *Nu1*ms mutations strengthen binding of terminase to *cosB* in complex I.

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