JOURNAL OF BACTERIOLOGY, Jan. 1999, p. 218–224 Vol. 181, No. 1
0021-9193/99/$04.00+0

Mutations That Extend the Specificity of the Endonuclease Activity of λ Terminase

JEAN SIPPY ARENS,* QI HANG, YOUNG HWANG,† BILL TUMA, SARA MAX, AND MIKE FEISS

Department of Microbiology, University of Iowa, Iowa City, Iowa 52242

Received 18 May 1998/Accepted 20 October 1998

Terminase, an enzyme encoded by the *Nu1* and *A* genes of bacteriophage lambda, is crucial for packaging concatemeric DNA into virions. *cosN*, a 22-bp segment, is the site on the virus chromosome where terminase introduces staggered nicks to cut the concatemer to generate unit-length virion chromosomes. Although *cosN* is rotationally symmetric, mutations in *cosN* have asymmetric effects. The *cosN* G_2C mutation (a G-to-C change at position 2) in the left half of *cosN* reduces the phage yield 10-fold, whereas the symmetric mutation *cosN* $C_{11}G$, in the right half of *cosN*, does not affect the burst size. The reduction in phage yield caused by *cosN* G_2C is correlated with a defect in *cos* cleavage. Three suppressors of the *cosN* G_2C mutation, *A-*$E_{515}G$, *A-*$N_{509}K$, and *A-*$R_{504}C$, have been isolated that restore the yield of λ *cosN* G_2C to the wild-type level. The suppressors are missense mutations that alter amino acids located near an ATPase domain of gpA. λ *A-*$E_{515}G$, *A-*$N_{509}K$, and *A-*$R_{504}C$ phages, which are *cosN*$^+$, also had wild-type burst sizes. In vitro *cos* cleavage experiments on *cosN* G_2C $C_{11}G$ DNA showed that the rate of cleavage for *A-*$E_{515}G$ terminase is three- to fourfold higher than for wild-type terminase. The *A-*$E_{515}G$ mutation changes residue 515 of gpA from glutamic acid to glycine. Uncharged polar and hydrophobic residues at position 515 suppressed the growth defect of λ *cosN* G_2C $C_{11}G$. In contrast, basic (K, R) and acidic (E, D) residues at position 515 failed to suppress the growth defect of λ *cosN* G_2C $C_{11}G$. In a λ *cosN*$^+$ background, all amino acids tested at position 515 were functional. These results suggest that *A-*$E_{515}G$ plays an indirect role in extending the specificity of the endonuclease activity of λ terminase.

λ is a double-stranded DNA phage with a 48.5-kb genome. The packaging of λ DNA into a preformed empty shell, the prohead, requires the phage-encoded enzyme terminase. Terminase is a heteromultimer consisting of gpNu1 (181 residues) and gpA (641 residues). Both subunits contain primary sequences which are characteristic of ATPases (Fig. 1) (22, 25, 45), and kinetic studies demonstrate that the holoenzyme hydrolyzes ATP and dATP (23, 31, 44). It has been found that each of the terminase subunits possesses an ATPase activity (4, 37). gpNu1 contains a DNA-stimulated, low-affinity site with a K_m of 469 μM and a k_{cat} of 84 min^{-1}. gpA contains a high-affinity site with a K_m of 4.6 μM and a k_{cat} of 38 min^{-1} (4, 30, 43). DNA lowers the K_m and increases the k_{cat} of the gpNu1 ATPase three- and twofold, respectively (43).

At its amino terminus, gpNu1 contains a putative helix-turn-helix (HTH) DNA binding motif which is thought to interact with *cosB*, the terminase binding site (Fig. 1) (18, 35). Adjacent to the HTH is the putative ATPase center. The carboxy half of gpNu1 has been shown to contain a domain for interaction with the amino-terminal 48 residues of gpA (21). In the C-terminal third of gpA are the putative ATPase center and a putative basic leucine zipper motif (17). The C-terminal 32 amino acids of gpA contain a specificity domain for interaction with the prohead (Fig. 1) (20, 47, 52).

The packaging substrate is a concatemer, or end-to-end multimer, of λ chromosomes which is generated at late times after infection. The site containing the DNA packaging signals is called *cos*. *cos* consists of three segments, *cosQ*, *cosN*, and *cosB* (Fig. 2). The first subsite, *cosQ*, is required for termination of packaging (13). The second segment, *cosN*, is the site where terminase introduces staggered nicks to generate the 12-base cohesive ends of virion DNA. *cosN* contains a 16-bp segment showing partial twofold rotational symmetry; the right half-site is called cosNR, and the left half-site is called cosNL (Fig. 2). The twofold rotational symmetry of *cosN* suggests that symmetrically disposed gpA subunits are responsible for nicking cosNL and cosNR. The third segment, *cosB*, contains three binding sites for gpNu1, called R3, R2 and R1 (40), and a site, I1, for integration host factor (IHF), a DNA binding-bending protein of the host, *Escherichia coli* (2, 33, 48, 49). IHF introduces a sharp bend in *cosB* between R3 and R2 (Fig. 2) (33), and packaging of λ DNA is enhanced threefold by IHF (24, 49). It is believed that IHF facilitates cooperative interactions between terminase bound to R3 and terminases bound to R2 and R1. A second DNA binding-bending protein of the host, HU, has also been proposed to play a role in gpNu1-*cos* interactions (36). Adjacent to *cos* are the terminase genes *Nu1* and *A* (Fig. 2).

An initial binding step of terminase to λ DNA is followed by nicking at cosNR and cosNL, in that order. Nicking is stimulated by the presence of ATP (12, 29), but ATP hydrolysis is not required (29). Cue and Feiss (12) found that the presence of ATP does not affect the affinity of terminase for DNA and concluded that ATP increases the velocity of the nicking reaction. The first nick is on the bottom strand, at position N1 in cosNR, and the second nick is on the top strand, at N2 in cosNL (Fig. 2) (29). The nicked strands are then separated, a step which requires ATP hydrolysis (29, 39). After separation of the cohesive ends, a stable complex forms, called Complex I, which consists of terminase bound to the left end of the chromosome (3, 34, 41), presumably through gpNu1-*cosB* interactions (12, 40). Terminase of Complex I then binds a prohead to form Complex II, a DNA-terminase-prohead ternary complex, and packaging proceeds. Complex II formation is facilitated by gpFI of λ (5, 16).

Mutations in *cosN*, specifically those in cosNL, affect the

* Corresponding author. Mailing address: 3-301G, BSB, University of Iowa, Iowa City, IA 52242. Phone: 319 335 8866. Fax: 319 335 9006. E-mail: jean-sippy@uiowa.edu.

† Present address: Infectious Disease Laboratory, The Salk Institute, La Jolla, CA 92037-1099.

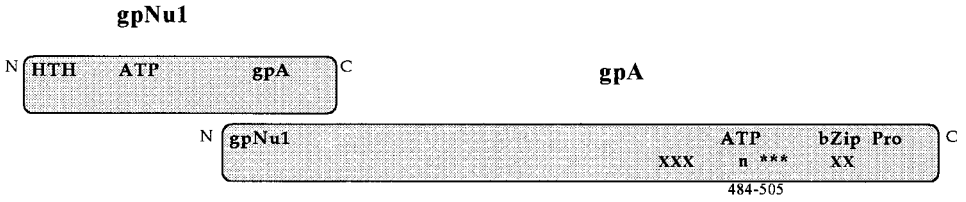

FIG. 1. Linear map of domains in gpNu1 and gpA. Relative positions of mutations which affect the endonucleolytic activity of terminase are shown for gpA. *, locations of the *A-$E_{515}G$*, *A-$N_{509}K$*, and *A-$R_{504}C$* mutations in the carboxy half of gpA; x, site of an altered amino acid that renders gpA deficient in nicking activity (17); n, residue 497, the site of the *A-$K_{497}D$* mutation (31); ATP, a proposed ATPase center; bZip, the putative basic leucine zipper region; Pro, the specificity domain for binding to proheads. Residues 484 to 505 comprise the Walker A segment of a putative ATPase domain in gpA. The proposed P-loop region of this domain encompasses residues 485 to 497 (25, 45). In gpNu1, HTH indicates the position of a proposed DNA binding HTH motif. gpNu1 and gpA interact via their carboxy and amino termini, respectively, to form the terminase heteromultimer.

nicking activity of terminase; one example is the *cosN* G_2C mutation (Fig. 2). *cosN* G_2C reduces the burst size of λ 10-fold due to a defect in the *cos* cleavage step of packaging (51). The G_2C and $C_{11}G$ mutations are rotationally symmetric (Fig. 2). The burst size of λ *cosN* G_2C $C_{11}G$ is threefold less than that of λ *cosN* G_2C (51). The *cosN* $C_{11}G$ mutation alone has no significant effect on the λ burst size or on *cos* cleavage in vitro. In vitro *cos* cleavage experiments of these and other rotationally symmetric mutants show that defects in the cosNL sequence are more detrimental to the cleavage activity of terminase than are defects in the cosNR sequence. λ *cosN* G_2C and λ *cosN* G_2C $C_{11}G$ mutants form minute plaques on an IHF^+ host strain and do not form plaques on an IHF^- strain. We describe here suppressors of the *cosN* G_2C $C_{11}G$ defect that have been found in pseudorevertants of λ *cosN* G_2C $C_{11}G$.

MATERIALS AND METHODS

Strains and plasmids. A list of the phages, *E. coli* strains, and plasmids used is given in Table 1, along with relevant markers and references.

Sequence and amino acid designations. The numbering convention of Daniels et al. (15) is used; numbering of the λ genome sequence begins with the first base of the left cohesive end (+1) and continues along the l strand (top strand as shown in Fig. 2) in a 5′ to 3′ direction. Base pair 48,502 is referred to as −1. The N1 nick position on the top strand is between bp −1 and 1. The position of the restriction enzyme cleavage sites is given as the first 5′ nucleotide of the recognition sequence. Amino acids are identified by the single-letter designation and numbered according to their position in the open reading frame of the protein.

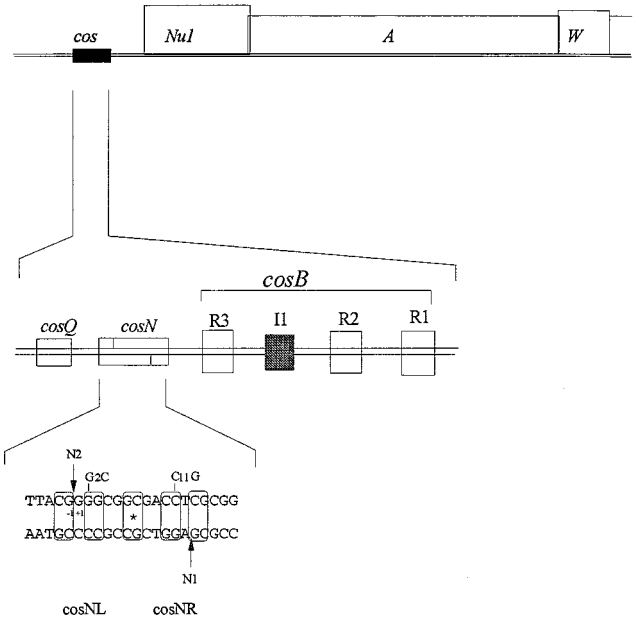

FIG. 2. The left end of the λ chromosome and expanded views of *cosB* and *cosN*. *Nu1* and *A*, the terminase genes, lie downstream from *cos*. *cos* consists of three subsites, *cosQ*, *cosN*, and *cosB*. *cosB* contains the terminase recognition sequences R3, R2, and R1 and the IHF binding site I1. The 22-bp sequence of *cosN* has partial rotational symmetry (boxed) around a central point (*), which divides *cosN* into cosNL and cosNR. The arrows show the positions of the nicks, N_1 and N_2, placed by terminase. Base pair 48,502 of the λ sequence is labeled −1.

Media. Tryptone broth (TB), TB plates, and TB soft agar were prepared as described in Arber et al. (1), except that each was supplemented with 0.01 M $MgSO_4$. Luria-Bertani (LB) broth and LB plates, both without glucose, were also as described in Arber et al. (1). Maltose was added to media at a final concentration of 0.4%, and ampicillin (AMP) and kanamycin (KAN) were added at concentrations of 100 and 50 μg/ml, respectively.

Enzymes, reagents, radiolabel, and DNA manipulations. Restriction enzymes, T4 DNA ligase, and Vent DNA polymerase were purchased from New England Biolabs. Boehringer Mannheim supplied ATP and the Random-Primed DNA Labeling Kit used to prepare radiolabeled probe for the Southern blot analyses. Ampli*Taq* DNA polymerase was purchased from Perkin-Elmer, and [α-^{32}P]dCTP was obtained from Amersham Life Science, Inc. Proteinase K, spermidine, putrescine, and β-mercaptoethanol were purchased from Sigma Chemical Co. All enzymes were used according to the manufacturer's directions. DNA was transformed into cells made competent with 0.1 M $CaCl_2$ (26). DNA sequencing was performed by the University of Iowa DNA Core Facility by using dye terminator cycle sequencing chemistry with Ampli*Taq* DNA polymerase and FS enzyme. Sequence analysis was done using a 373A Stretch Fluorescent Automated Sequencer (Applied Biosystems).

DNA preparations, PCR, and primers. λ *cosN* G_2C $C_{11}G$ *gal*$^+$ *att*$^+$ *cI*857 *A-$E_{515}G$* DNA was prepared by phenol extraction of CsCl-purified phage lysate as described by Arber et al. (1). Plasmid DNA was prepared with commercial kits (Qiagen, Inc.) or by the method of Birnboim and Doly (6). Diagnostic PCRs were performed on phage DNA supplied in 10 μl of crude lysate under the following conditions: 4.5 mM $MgCl_2$, 0.1% Tween 20, 200 μM (each) dNTP, 0.5 μM (each) primer, 2.5 U of Ampli*Taq* DNA polymerase, and amplification buffer supplied by the manufacturer. The reactions were run for 30 cycles of 95°C for 1 min, 55°C for 45 s, and 72°C for 1 min, followed by one 7-min extension cycle at 72°C. DNA for cloning was prepared by PCR with the following reaction mixture: 10 μl of crude lysate, 6.0 mM $MgSO_4$, 400 μM (each) dNTP, 0.5 μM (each) primer, 2.0 U of Vent DNA polymerase, and amplification buffer supplied by the manufacturer. The reactions were run as above, except that the annealing temperature was 60 rather than 55°C. Sequencing primers and PCR primers were prepared on a four-column Applied Biosystems DNA synthesizer.

Crosses. Phage versus plasmid crosses were done with lysogens transformed with plasmid DNA and selected on L-agar plates containing AMP or AMP and KAN (for λ-P1 lysogens) at 31°C. Overnight cultures of the transformed lysogens were diluted 1:100 in LB broth and grown to 5×10^7 cells/ml at 31°C. Induction of prophages at 42°C for 20 min was followed by incubation at 37°C for 60 min. The lysates were treated with chloroform, clarified by centrifugation, and plated on appropriate cells for viable recombinants. Standard phage crosses were performed as described by Arber et al. (1).

***mutD* mutagenesis.** Phage lysates were grown on MF2449 as described by Fowler et al. (19). Mutant phages were isolated on MF1972.

Terminase preparation and in vitro *cos* cleavage reactions. Crude extracts of wild-type and *A-$E_{515}G$* terminases were prepared by sonication of cells from induced cultures of MF1427[pCM101] and OR1265[pCM101 *A-$E_{515}G$*], respectively, according to the method of Chow et al. (9). Wild-type and *A-$E_{515}G$* terminases were purified by a modification (30) of the method of Tomka and Catalano (44).

cos$^+$ and *cosN* G_2C $C_{11}G$ DNA substrates were purified from cosmid pSX1, which carries the wild-type *cos* segment and its derivative plasmid pUC19 *cosN* G_2C $C_{11}G$. The plasmids were linearized with *Bsa*I and yield 1.51- and 1.91-kb fragments when cut at *cos* by terminase. The *cos* cleavage assay was performed by using the protocol of Chow et al. (9); the 20-μl reactions mixtures contained 30 mM Tris-HCl (pH 9.0), 10 mM $MgCl_2$, 3 mM spermidine, 6 mM putrescine, 7 mM β-mercaptoethanol, 1.5 mM EDTA, 1.5 mM ATP, 10 nM IHF, and 150 nM purified wild-type or *A-$E_{515}G$* terminase. After incubation at room temper-

TABLE 1. Strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Reference or source
Phages		
λ *cosN* G_2C $C_{11}G$ *att*$^+$ *gal*$^+$ *cI*857	*cosN* G_2C $C_{11}G$	51
λ *A*am515 cI857	Used for residue 515 substitution studies	This work
λ *A*am11am32 *cI*857	*cos*$^+$ *A*am515, *A-*$R_{504}C$, *A-*$N_{509}K$, and *A-*$E_{515}G$ phage constructions	20
λ-P1:5R Kn^r *cI*857	Used for rescues and burst size studies	37
E. coli C strains		
MF1427	C1a *galK sup*^{0}	42
MF1966	C-4514 *supE*	32
MF1968	C-4518 *supF*	32
MF1972	C1a *sup*^{0} *himA*::Tn10 *hip*::Cm^r IHF$^-$ strain	10
MF2449	C1a *mutD*	19
MF2548	C-1055	46
E. coli K strains		
XL-1 Blue	Host for cloning vectors *supE*	Stratagene Cloning Systems
OR1265	Host for terminase expression plasmids	9
Plasmids		
pIBI30, pIBI31	Cloning vectors	International Biotechnologies, Inc.
pBluescript II SK$^-$	Cloning vector	Stratagene Cloning Systems
pTSNA-*A*$^+$, -*A-*$R_{504}C$, -*A-*$N_{509}K$, or -*A-*$E_{515}G$	Used for *cosB* and *cosN* rescues; λ *cos*$^+$ *A-*$R_{504}C$, *A-*$N_{509}K$, and *A-*$E_{515}G$ constructions; λ DNA 732-2556 in pBluescript	This work
pRV-*Nu1*$^+$, -*Nu1*ms1, -ms2, or -ms3	Used for *cosB* and *cosN* rescues; λ DNA 194-2819 in pIBI31	11
pSX1	*cos*$^+$ cleavage substrate; probe for Southern analyses; λ DNA 47942-194 in pUC19	51
pUC19 *cosN*$_i$	*cos* cleavage substrate; i = G_2C $C_{11}G$, G_3C $C_{10}G$, or $A_{-1}T$ $T_{13}A$; λ DNA 47942-194 in pUC19	51
pCM101	Wild-type terminase expression plasmid	9
pCM101 *A-*$E_{515}G$	*A-*$E_{515}G$ terminase expression plasmid	29a
pE_{515}X	Used for residue 515 substitutions: X = D, E, K, R, Q, S, A, F, G, I, V, or Y; λ DNA 2218 to 2815 in pIBI30	29a
pHW473	λ *cos*$^+$ *A*am515 construction; am mutation at residue 515; λ DNA 48473–5505 in pIBI30	29a

ature, 2 μl of agarose gel loading buffer (50% glycerol, 0.1 M EDTA, 1% sodium dodecyl sulfate, and 0.1% bromophenol blue) was added to stop the reaction.

The samples were heated at 65°C for 10 min and subjected to 1% agarose gel electrophoresis. Following electrophoresis, the DNA was transferred onto a GeneScreen Plus (New England Nuclear) membrane. DNA hybridization was performed with radiolabeled pSX1 as probe. The extent of cleavage was determined by scanning with the Packard InstantImager (Packard Instrument Co., Downers Grove, Ill.) scanning apparatus.

RESULTS

Isolation and mapping of *cosN* G_2C $C_{11}G$ suppressors. λ *cosN* G_2C $C_{11}G$ *gal*$^+$ *att*$^+$ *cI*857 was mutagenized by infecting MF2449 (*mutD*), and the resulting lysate was plated on MF1972 (IHF$^-$). Plaque-forming variants were found at a frequency of 2×10^{-5}/PFU; nine revertants were chosen for study. The *cosN* G_2C mutation creates an *Hha*I site. Phage DNA was amplified by PCR, and the presence of the *cosN* G_2C mutation was verified by cutting with *Hha*I. Chromosomal DNA was prepared from one of the isolates and cut with *Mlu*I, and the fragments were individually cloned into pIBI30. To map the suppressor, plasmids containing *Mlu*I fragments were used in marker rescue experiments as follows. The plasmids were used to transform MF1427 (λ *cosN* G_2C $C_{11}G$ *gal*$^+$ *att*$^+$ *cI*857) to Ap^r. The resulting plasmid-bearing lysogens were induced, and the lysates were plated to identify recombinants able to plate on the IHF$^-$ strain MF1972. The control cross yielded $<10^{-6}$ PFU/viable cell, whereas the cross with the fragment containing bp 458 to 5548 produced 10^{-1} PFU/viable cell (Table 2). This fragment carrying the suppressor was also used in a similar cross with the single *cosN* mutant, λ *cosN* G_2C; *cosN* G_2C was also suppressed by this fragment (results not shown). The fragment containing the suppressor was then subcloned for further marker rescue crosses, and the suppressor was mapped to the segment extending from bp 2212 to

TABLE 2. Suppression of the *cosN* G_2C mutation: crosses between λ *cosN* G_2C $C_{11}G$ and cloned DNA segments of λ *A*$^+$, λ *A-*$E_{515}G$, λ *A-*$N_{509}K$, or λ *A-*$R_{504}C$[a]

Sequence of cloned segment (bp)	Allele	Amino acid change(s) in gpA	PFU/viable cell
1818–2556	*A*$^+$	None	$<5.0 \times 10^{-6}$
458–5548	*A-*$E_{515}G$	$E_{515}G$	1.1×10^{-1}
2212–2847	*A-*$N_{509}K$	$N_{509}K$	4.1×10^{-3}
2190–2556	*A-*$R_{504}C$	$R_{504}C$	7.7×10^{-4}[b]
1818–2556	*A-*$R_{504}C$	$P_{416}S$, $R_{504}C$	1.9×10^{-3}[b]
1818–2190		$P_{416}S$	$<5.3 \times 10^{-5}$[b]

[a] Crosses were performed with plasmids in an MF1427 (λ *cosN* G_2C $C_{11}G$ *gal*$^+$ *att*$^+$ *cI*857) lysogen and were plated on MF1972 for plaques. The higher frequency of recombinants for *A-*$E_{515}G$ is a reflection of the larger λ DNA insert in the plasmid used (5 kb).

[b] These lysates were plated on MF1427 for recombinants forming normal-sized plaques.

2815. Sequencing revealed a transition mutation, $A_{2254}G$, that resulted in changing codon 515 of the *A* gene from the glutamic acid codon GAA to the glycine codon GGA; $A_{2254}G$ also creates a new *Hin*fI site. The $A_{2254}G$ suppressor mutation was designated *A-E*$_{515}$*G*. For each of the eight remaining isolates, the segment of phage DNA from bp 2212 to 2815 was amplified by PCR and screened by restriction analysis for the *Hin*fI site. Of these eight isolates, seven were *Hin*fI$^+$ and were presumed to be identical to *A-E*$_{515}$*G*. To map the suppressor of the eighth isolate, the DNA segment extending from bp 1818 to 2556 was amplified by PCR and cloned. Marker rescue crosses showed the cloned segment contained a suppressor of *cosN* G_2C $C_{11}G$ (Table 2). Sequencing of the segment revealed two mutations, one at bp 1958 (CCG to CCT, $P_{416}S$) and one at bp 2220 (CGT to TGT, $R_{504}C$). These two mutations were separated by subcloning by using the *Acc*I site at bp 2190, and the resulting plasmids were used in a phage versus plasmid cross with λ *cosN* G_2C $C_{11}G$ *gal*$^+$ *att*$^+$ *cI*857. The mutation at bp 1958 ($P_{416}S$) did not suppress *cosN* G_2C $C_{11}G$, whereas the mutation at bp 2220 ($R_{504}C$) did (Table 2). The mutation at bp 2220 ($R_{504}C$) was named *A-R*$_{504}$*C*.

A second lysate of λ *cosN* G_2C $C_{11}G$, unmutagenized, was plated on MF1972, and plaque-forming variants were found at a frequency of 2×10^{-7}/PFU. Six variants were picked for further study. Five of the phages contained a true reversion of the *cosN* G_2C mutation, based on restriction analysis of phage DNA amplified by PCR. DNA from the sixth revertant was amplified by PCR (bp 2212 to 2847), cloned into pBluescript, and shown to contain a suppressor of *cosN* G_2C $C_{11}G$ (Table 2). A transversion was found at bp 2237 (AAC to AAG, $N_{509}K$); this suppressor was named *A-N*$_{509}$*K*.

The *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* mutations allowed λ *cosN* G_2C $C_{11}G$ to form normal-sized plaques on MF1427 and small plaques on MF1972.

Can the *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* mutations suppress *cosB* mutations? We next asked if the *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* suppressors could rescue a *cosB* defect by using phage versus plasmid crosses. The prophage used was λ-P1:5R *cI*857 Kn^r *cosB* R3$^-$R2$^-$R1$^-$. *cosB* R3$^-$R2$^-$R1$^-$ contains a transition mutation in each of the R sequences of *cosB* (Fig. 2); phages with these mutations are unable to form plaques (10). Plasmids used were the pTSNA series, which contain λ DNA inserts from bp 732 to 2556 and lack the *Nu1* gene but contain the *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, *A-R*$_{504}$*C*, or *A*$^+$ alleles. The control plasmids were the pRV series, containing λ DNA from bp 194 to 2819, which includes the *Nu1* and *A* genes and the *Nu1*ms1, *Nu1*ms2, *Nu1*ms3, or *Nu1*$^+$ alleles. The *Nu1*ms1, *Nu1*ms2, and *Nu1*ms3 mutations are suppressors of *cosB* defects (11). Lysates from the crosses were plated on MF1427; the results are shown in Table 3. The crosses with the positive controls pRV5, pRV39, and pRV7 produced approximately 10^4 to 10^6 PFU/ml, whereas crosses with pTSNA *A-E*$_{515}$*G*, pTSNA *A-N*$_{509}$*K*, and pTSNA *A-R*$_{504}$*C* produced 10 to 25 PFU/ml. The crosses with the negative controls, pTSNA and pRV12, produced 10 and 15 PFU/ml, respectively. The *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* mutations were unable to suppress the *cosB* R3$^-$R2$^-$R1$^-$ defect.

Can the *cosN* G_2C $C_{11}G$ defect be suppressed by the *cosB* suppressors *Nu1*ms1, *Nu1*ms2, and *Nu1*ms3? We also asked the reciprocal question, whether suppressors of a *cosB* defect could suppress a *cosN* defect. Again, phage versus plasmid crosses were done; the prophage used was λ-P1:5R *cI*857 Kn^r with the *cosN* G_2C $C_{11}G$ alleles. The plasmids were the pTSNA and pRV series described above, and cross lysates were plated on MF1427 for healthy plaques. Results are shown in Table 3. The yield of PFU in the crosses with pRV5, pRV39, and pRV7, which contain *cosB* suppressors, was as low as in the crosses with the negative controls pRV12 and pTSNA. The *Nu1*ms1, *Nu1*ms2, and *Nu1*ms3 mutations did not suppress the *cosN* G_2C $C_{11}G$ defect.

TABLE 3. Suppression of *cosB* or *cosN* mutations: crosses between λ *cosB* or λ *cosN* and plasmids bearing various *Nu1* or *A* alleles[a]

Phage	Plasmid	Allele	PFU/ml
λ-P1 *cosB* R3$^-$ R2$^-$ R1$^-$			
	pTSNA *A-E*$_{515}$*G*	*A-E*$_{515}$*G*	10
	pTSNA *A-N*$_{509}$*K*	*A-N*$_{509}$*K*	15
	pTSNA *A-R*$_{504}$*C*	*A-R*$_{504}$*C*	25
	pTSNA	*A*$^+$	10
	pRV5	*Nu1*ms1	7.6×10^5
	pRV39	*Nu1*ms2	2.0×10^4
	pRV7	*Nu1*ms3	1.5×10^5
	pRV12	*Nu1*$^+$	15
λ-P1 *cosN* G_2C $C_{11}G$			
	pRV5	*Nu1*ms1	3.9×10^2
	pRV39	*Nu1*ms2	5.5×10^2
	pRV7	*Nu1*ms3	7.7×10^2
	pRV12	*Nu1*$^+$	4.5×10^2
	pTSNA *A-E*$_{515}$*G*	*A-E*$_{515}$*G*	9.1×10^5
	pTSNA *A-N*$_{509}$*K*	*A-N*$_{509}$*K*	4.7×10^5
	pTSNA *A-R*$_{504}$*C*	*A-R*$_{504}$*C*	1.6×10^5
	pTSNA	*A*$^+$	1.5×10^3

[a] Phages are in a λ-P1:5R *cI*857 Kn^r background. Crosses were performed by inducing plasmid-bearing MF1427 cells lysogenized with *cosB* R3$^-$R2$^-$R1$^-$ or *cosN* G_2C $C_{11}G$ phages. The cross lysates were plated on MF1427. The pRV5, pRV39, and pRV7 plasmids are called rev1, rev9, and rev6, respectively, in reference 11.

Does λ *cosN*$^+$ *A-E*$_{515}$*G*, *-A-N*$_{509}$*K*, or *-A-R*$_{504}$*C* form a plaque? Since the *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* mutations were specific to *cosN*, we asked if they were specific to *cosN* G_2C $C_{11}G$ by trying to construct λ *cosN*$^+$ *A-E*$_{515}$*G*, *-A-N*$_{509}$*K*, or *-A-R*$_{504}$*C*. We crossed the *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* mutations into λ *cosN*$^+$. Cells containing pTSNA *A-E*$_{515}$*G*, *-A-N*$_{509}$*K*, or *-A-R*$_{504}$*C* were infected with λ *A*am11 am32 *cI*857. am$^+$ recombinants were found when the resulting lysate was plated on MF1427 (*sup*^{0}). DNA from the am$^+$ recombinants was amplified by PCR, and sequence analysis of the PCR product confirmed the presence of the *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* mutations. Thus λ *cosN*$^+$ is viable with *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C*.

Burst size studies. A set of phages in the λ-P1:5R *cI*857 Kn^r background was constructed to enable us to examine burst sizes in the presence and absence of IHF. Burst sizes were determined by induction of prophages in either MF1427 (IHF$^+$) or MF1972 (IHF$^-$) (Table 4). The phages were made by phage versus plasmid crosses or by standard phage crosses. The *cosN* G_2C $C_{11}G$ *A*$^+$ phage had a smaller burst size and was more responsive to IHF than the wild-type phage. The burst size of the wild-type phage increased about 3-fold in MF1427 (IHF$^+$) cells, whereas the burst of the *cosN* G_2C $C_{11}G$ phage increased about 10-fold (Table 4). With *A-E*$_{515}$*G* present, the *cosN* G_2C $C_{11}G$ phage's burst size was restored to wild-type levels in both MF1427 and MF1972 (Table 4). The burst size of λ *cosN* G_2C $C_{11}G$ in IHF$^+$ cells (MF1427) was also restored to near wild-type levels with *A-N*$_{509}$*K* and *A-R*$_{504}$*C*. The burst sizes of λ *cosN* G_2C $C_{11}G$ *A-N*$_{509}$*K* and λ *cosN* G_2C $C_{11}G$ *A-R*$_{504}$*C* were 10- and 4-fold less than that of λ *cosN*$^+$ *A*$^+$ in IHF$^-$ cells, respectively (Table 4).

The burst sizes of the λ *cosN*$^+$ *A-E*$_{515}$*G*, *-A-N*$_{509}$*K*, and *-A-R*$_{504}$*C* phages were near wild-type levels in both MF1427 and MF1972 (Table 4). Thus, the *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* suppressors do not diminish the burst size of λ *cosN*$^+$.

TABLE 4. Burst sizes of phages with various *cosN* and *A* alleles normalized to λ *cosN*$^+$ *A*$^+$[a]

Genotype	Burst size (PFU/cell) in:	
	MF1427	MF1972
cosN$^+$ *A*$^+$	1.0	0.29
cosN $G_2C\ C_{11}G$ *A*$^+$	0.01[b]	8.2×10^{-4}
cosN $G_2C\ C_{11}G$ *A-E*$_{515}$*G*	0.83	0.18
cosN $G_2C\ C_{11}G$ *A-N*$_{509}$*K*	0.69	0.03
cosN $G_2C\ C_{11}G$ *A-R*$_{504}$*C*	0.43	0.07
cosN$^+$ *A-E*$_{515}$*G*	1.01	0.23
cosN$^+$ *A-N*$_{509}$*K*	0.60	0.29
cosN$^+$ *A-R*$_{504}$*C*	0.38	0.25

[a] λ-P1:5R Kn^r *cI*857 lysogens in MF1427 (IHF$^+$) and MF1972 (IHF$^-$) were induced and plated on MF1427. Values are PFU/induced cell, normalized to λ *cosN*$^+$ *A*$^+$ induced from MF1427, and are the averages of three experiments. The average burst of λ *cosN*$^+$ *A*$^+$ on MF1427 was 243 and that on MF1972 was 71.6.

[b] These lysates were plated on MF2548 to reduce the background of revertants which arise spontaneously on MF1427.

Comparison of the *cos* cleavage activities of wild-type and *A-E*$_{515}$*G* terminases. Since it had previously been shown that the *cosN* $G_2C\ C_{11}G$ mutations affect *cos* cleavage (51), we asked if the *A-E*$_{515}$*G* mutation improved the ability of terminase to cut DNA containing the *cosN* $G_2C\ C_{11}G$ mutations. *A-E*$_{515}$*G* was cloned into the terminase expression vector pCM101 (9). Preliminary *cos* cleavage reactions with crude terminase extracts showed that *A-E*$_{515}$*G* terminase was able to efficiently cut several *cosN* substrates, including *cosN*$^+$, *cosN* $G_3C\ C_{10}G$, *cosN* $A_{-1}T\ T_{13}A$, and *cosN* $G_2C\ C_{11}G$ (results not shown). To examine the kinetics of *A-E*$_{515}$*G* terminase, purified wild-type and *A-E*$_{515}$*G* terminases were used in *cos* cleavage reactions with *cosN*$^+$ and *cosN* $G_2C\ C_{11}G$ substrate DNAs. With *cosN*$^+$ DNA, the initial rates of cleavage are similar for both terminases over a range of DNA substrate concentrations (Fig. 3a). In reactions with the *cosN* $G_2C\ C_{11}G$ substrate, the *A-E*$_{515}$*G* terminase shows a faster rate of cleavage than the wild-type terminase (Fig. 3b). Thus, the *A-E*$_{515}$*G* mutation significantly improved the ability of terminase to cleave *cosN* $G_2C\ C_{11}G$ DNA.

Changing gpA residue 515 in λ *cosN*$^+$ and λ *cosN* $G_2C\ C_{11}G$. To learn about the effects of amino acid substitutions at the residue affected by *A-E*$_{515}$*G*, the pE$_{515}$X series of plasmids were used in phage versus plasmid crosses. The plasmid inserts are λ DNA from bp 2218 to 2815 and contain various replacements at residue 515 of gpA (29a). Phages containing either *cosN*$^+$ or *cosN* $G_2C\ C_{11}G$ were crossed with each of the plasmids. To test the effects of the substitutions in λ *cosN*$^+$, crosses were done in MF1966 (*supE*) (λ *A*am515 *cI*857) cells, and the lysates were plated for am$^+$ recombinants on MF1972 (*sup*^{0}). λ *A*am515 *cI*857 was constructed by a phage versus plasmid cross in MF1968 (*supF*) cells by using pHW473, which contains the *A*am515 marker, and λ *A*am11 am32 *cI*857, which will not grow on a *supE* host; the cross lysate was plated on MF1966 (*supE*). The *A*am515 change results in a new *Bfa*I site, which was verified by restriction analysis of DNA generated by PCR. To test the effects of the substitutions in λ *cosN* $G_2C\ C_{11}G$, crosses were done in MF1427 (λ *cosN* $G_2C\ C_{11}G$ *gal*$^+$ *att*$^+$ *cI*857) cells, and the lysates were plated for viable phage on MF1972 (IHF$^-$). Residues at position 515 which are acidic, basic, polar uncharged, and hydrophobic allowed plaque formation of λ *cosN*$^+$ (Table 5). In contrast, only polar uncharged and hydrophobic residues allowed growth of λ *cosN* $G_2C\ C_{11}G$; acidic and basic residues at position 515 of gpA did not allow plaque formation. Further, all of the plaque-forming recombinants of λ *cosN* $G_2C\ C_{11}G$ formed plaques at 31, 37, and 42°C on MF1427.

DISCUSSION

The *A-E*$_{515}$*G*, *A-N*$_{509}$*K* and *A-R*$_{504}$*C* mutations were isolated as suppressors of the *cosN* $G_2C\ C_{11}G$ mutations. A number of *cos* mutations, including *cosN* $G_2C\ C_{11}G$, cause plaque formation to be dependent on IHF (reviewed in reference 8). Based on these previous results, we isolated suppressors of the *cosN* $G_2C\ C_{11}G$ mutations by isolating variants of λ *cosN* $G_2C\ C_{11}G$ able to form plaques on a host lacking IHF. The suppressors were found to be missense mutations in the *A* gene. The suppressors result in normal and small-sized plaques by λ *cosN* $G_2C\ C_{11}G$ on cells with and without IHF, respectively.

cosN mutations affect *cos* cleavage in vitro, and the in vitro

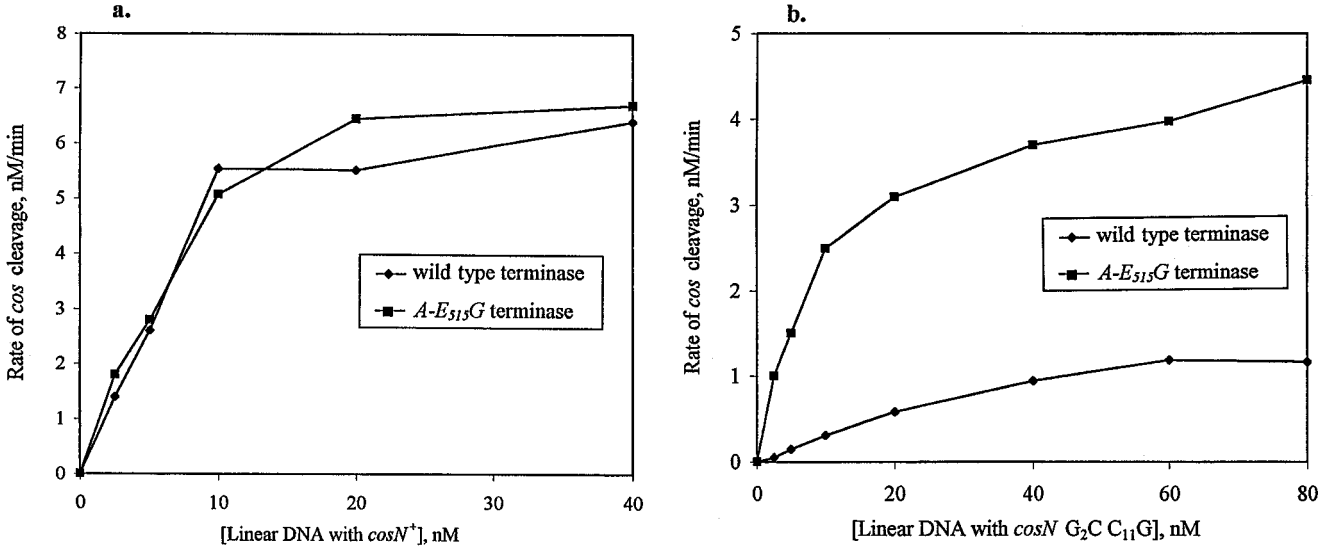

FIG. 3. *cos* cleavage rates of wild-type terminase and *A-E*$_{515}$*G* terminase. (a) *cos* cleavage on *cosN*$^+$ substrate. The reactions used *Bsa*I-linearized pSX1, a cosmid carrying *cosN*$^+$, as the DNA substrate. Wild-type terminase and *A-E*$_{515}$*G* terminase were used to cleave the DNA substrate in a range of concentrations. (b) *cos* cleavage on *cosN* $G_2C\ C_{11}G$ substrate. The reactions used a pSX1 derivative cosmid which carries *cosN* $G_2C\ C_{11}G$. This cosmid is linearized by *Bsa*I. Wild-type terminase and *A-E*$_{515}$*G* terminase were used to cleave the DNA substrate in a range of substrate concentrations. Other reaction conditions were as described in Materials and Methods.

TABLE 5. Effect of changing gpA residue 515 on viability of λ *cosN*$^+$ and λ *cosN* $G_2C\ C_{11}G$[a]

Group	Amino acid	Viable recombinants on MF1972 for:	
		cosN$^+$	*cosN* $G_2C\ C_{11}G$
Acidic	D	+	−
	E	+	−
Basic	K	+	−
	R	+	−
Polar uncharged	Q	+	+
	S	+	+
Hydrophobic	A	+	+
	F	+	+
	G	+	+
	I	+	+
	V	+	+
	Y	+	+

[a] Crosses were performed in an MF1966 (λ *A*am515 *cI*857) lysogen for the *cosN*$^+$ allele and in an MF1427 (λ *cosN* $G_2C\ C_{11}G$ *gal*$^+$ *att*$^+$ *cI*857) lysogen for the *cosN* $G_2C\ C_{11}G$ allele; both were plated on MF1972 for plaques.

effects of the mutations parallel effects on virus yield (27, 51). Since the in vitro *cos* cleavage reaction is an analog of the in vivo *cos* cleavage reaction that initiates packaging of a virus chromosome, the reduced virus yield caused by a *cosN* mutation is due at least in part to a defect in initial *cos* cleavage.

The nature of the *cosN* $G_2C\ C_{11}G$ defect is revealed by our kinetic studies with purified terminase; wild-type terminase has a reduced efficiency in cleaving *cosN* $G_2C\ C_{11}G$ (Fig. 3b). Considering that the *cosN* G_2C and $C_{11}G$ mutations are located in rotationally symmetric base pairs of *cosN*, it is likely that symmetrically disposed gpA subunits interact with *cosN* half-sites, and that the *cosN* G_2C and $C_{11}G$ mutations affect an interaction between gpA and the *cosN* half-site (39). The *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* suppressors must directly or indirectly affect the gpA-*cosN* interaction. We next discuss the nature of the suppressor mutations in the light of these possible mechanisms.

The suppressors map in a cluster, affecting gpA residues 504, 509, and 515. Other mutations which specifically alter the endonuclease activity of terminase also affect the C-terminal third of terminase as follows. Mutations affecting residues 401, 403, and 404 abolish the endonuclease activity of terminase; these mutations reside in an amino acid segment which shares homology with DNA polymerases and helicases. Similar loss-of-endonuclease mutations affect residues 586 and 600, located in the putative basic leucine zipper motif (Fig. 1) (17). Finally, a mutation which changes residue 497, which is located in the putative ATPase center of gpA, has been shown to abolish the endonuclease activity of terminase (Fig. 1) (31).

A detailed study of residue 515 and the *A-E*$_{515}$*G* suppressor was carried out. All of the 12 amino acids tested at position 515 were functional in a wild-type background, including acidic (D, E), basic (K, R), polar uncharged (Q, S), and hydrophobic (A, F, G, I, V, Y) sets. This lack of specificity suggests that residue 515 does not form specific interactions with *cosN*. A broad spectrum of residues at position 515 (polar uncharged and hydrophobic) suppress *cosN* $G_2C\ C_{11}G$ (Table 5), and the growth of λ *cosN* $G_2C\ C_{11}G$ with these substitutions is not temperature sensitive. These results suggest that residue 515 indirectly affects interactions of gpA with *cosN* and is not involved in interactions crucial to the integrity of gpA. Finally, *A-E*$_{515}$*G* terminase has a broadened substrate specificity for the cleavage reaction: *cosN*$^+$, *cosN* $G_3C\ C_{10}G$, *cosN* $A_{-1}T\ T_{13}A$, and *cosN* $G_2C\ C_{11}G$ are all nicked by *A-E*$_{515}$*G* terminase.

To discuss the effects of *A-E*$_{515}$*G* on *cos* cleavage we must consider the complex interactions of terminase with *cos* and with ATP. Terminase is a bivalent DNA binding enzyme; in addition to gpA-*cosN* interaction, gpNu1 interacts with *cosB* (7; reviewed in reference 8). This terminase-*cosB* interaction anchors terminase when *cos* cleavage occurs at *cosN* (Fig. 2), and likely decreases the dissociation rate of the overall terminase-*cos* interaction. The efficiency of *cosN* nicking by gpA is thereby increased. ATP binding by terminase apparently alters the conformation of terminase in a way that stimulates endonuclease activity and increases the fidelity of *cosN* nicking (12, 29). ATP does not increase the affinity of terminase for DNA (12). There is a putative ATPase center in gpA; the residues from 485 to 497 are proposed to comprise a P-loop, the glycine-rich, flexible, phosphate binding loop that is terminated by a lysine, which is proposed to be K_{497} in gpA (Fig. 1) (25). A lethal mutation causing residue 497 to be changed from K to D (*A-K*$_{497}$*D*) decreases the rate of *cos* cleavage about 2,000-fold from that of the wild type (31) and reduces the affinity of ATP by 14-fold (30). The interpretation of the *A-K*$_{497}$*D* mutation is that communication between the ATP-binding and endonuclease centers has been disrupted. Terminase with the *A-K*$_{497}$*D* change is defective in *cos* cleavage even at a saturating level of ATP (31).

A-E$_{515}$*G* terminase has an endonucleolytic activity that is different from that of wild-type terminase; it cuts a variety of *cosN* substrates and cuts *cosN* $G_2C\ C_{11}G$ more efficiently. The ATPase activity of the *A-E*$_{515}$*G* terminase also differs from that of the wild type, with K_m being increased twofold and k_{cat} being decreased threefold (29a). Further, *A-E*$_{515}$*G* is also a suppressor of the *A-K*$_{497}$*D* mutation. Studies show that the *cos* cleavage activity of the *A-K*$_{497}$*D A-E*$_{515}$*G* enzyme is ATP stimulated, indicating that the change caused by the *A-E*$_{515}$*G* mutation restores communication between the endonuclease and ATP-binding domains of *A-K*$_{497}$*D* terminase (29a). The ability to alter communication between the ATP and endonuclease centers enables *A-E*$_{515}$*G* terminase to cut *cosN* $G_2C\ C_{11}G$ more efficiently. Structural studies of *A-E*$_{515}$*G* terminase would increase our understanding of the communication.

It is interesting that the *Nu1*ms1, *Nu1*ms2, and *Nu1*ms3 mutations, which suppress *cosB* defects (11), do not suppress *cosN* $G_2C\ C_{11}G$. The *Nu1*ms suppressors were found to act at a postcleavage step of packaging, likely involving the formation or stability of DNA-terminase Complex I (7). The suppressors described in this paper affect gpA-*cosN* interactions. Recently, suppressors of the third *cos* subsite, *cosQ*, were described (14). The *cosQ* suppressors were also distinct; a mild *cosQ* mutation could be suppressed by slowing the rate of DNA packaging, a suppression mechanism quite distinct from the *cosB* and *cosN* suppression mechanisms.

We have shown that the poor growth of λ *cosN* $G_2C\ C_{11}G$ phage is suppressed by each of three mutations in the carboxy terminus of gpA and that one of them, *A-E*$_{515}$*G*, suppresses at the level of *cos* cleavage. A variety of mutations have been found to affect the endonuclease function of terminase, and the *A-E*$_{515}$*G*, *A-N*$_{509}$*K*, and *A-R*$_{504}$*C* mutations give further insights into the complexity of the endonucleolytic activity. The study of additional suppressors which are specific to *cosN* G_2C will allow us to determine specific *cosN*-terminase interactions.

ACKNOWLEDGMENTS

We thank our coworkers, David Cue, Carol Duffy, Hillary Johnson, Jenny Meyer, John Randell, Fyodor Tereshchenko, and Doug Wiec-

zorek, for advice and helpful discussions during the course of this work.

This research was supported by NIH research grant GM 51611.

REFERENCES

1. **Arber, W., L. Enquist, B. Hohn, N. E. Murray, and K. Murray.** 1983. Experimental methods for use with lambda, p. 433–466. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. **Bear, S., D. Court, and D. Friedman.** 1984. An accessory role for *Escherichia coli* integration host factor: characterization of a lambda mutant dependent upon integration host factor for DNA packaging. J. Virol. **52:**966–972.
3. **Becker, A., M. Marko, and M. Gold.** 1977. Early events in the *in vitro* packaging of bacteriophage λ DNA. Virology **78:**291–305.
4. **Becker, A., and M. Gold.** 1988. Prediction of an ATP reactive center in the small subunit, Nu1, of the phage lambda terminase enzyme. J. Mol. Biol. **199:**219–222.
5. **Becker, A., H. Murialdo, H. Lucko, and J. Morell.** 1988. Bacteriophage lambda DNA packaging. The product of the *FI* gene promotes the incorporation of the prohead to the DNA-terminase complex. J. Mol. Biol. **199:**597–607.
6. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
7. **Cai, Z.-H., Y. Hwang, D. Cue, C. Catalano, and M. Feiss.** 1997. Mutations in *Nu1*, the gene encoding the small subunit of bacteriophage λ terminase, suppress the postcleavage DNA packaging defect of *cosB* mutations. J. Bacteriol. **179:**2479–2485.
8. **Catalano, C. E., D. Cue, and M. Feiss.** 1995. Virus DNA packaging: the strategy used by phage lambda. Mol. Microbiol. **16:**1075–1086.
9. **Chow, S., E. Daub, and H. Murialdo.** 1987. The overproduction of DNA terminase of coliphage lambda. Gene **60:**177–229.
10. **Cue, D., and M. Feiss.** 1992. Genetic analysis of *cosB*, the binding site for terminase, the DNA packaging enzyme of bacteriophage λ. J. Mol. Biol. **228:**58–71.
11. **Cue, D., and M. Feiss.** 1992. Genetic analysis of mutations affecting terminase, the bacteriophage λ DNA packaging enzyme, that suppress mutations in *cosB*, the terminase binding site. J. Mol. Biol. **228:**72–87.
12. **Cue, D., and M. Feiss.** 1993. The role of *cosB*, the binding site for terminase, the DNA packaging enzyme of bacteriophage λ, in the nicking reaction. J. Mol. Biol. **234:**594–609.
13. **Cue, D., and M. Feiss.** 1993. A site required for termination of packaging of the phage λ chromosome. Proc. Natl. Acad. Sci. USA **90:**9290–9294.
14. **Cue, D., and M. Feiss.** 1997. Genetic evidence that recognition of *cosQ*, the signal for termination of phage λ DNA packaging, depends on the extent of head filling. Genetics **147:**7–17.
15. **Daniels, D., J. Schroeder, W. Szybalski, F. Sanger, A. Coulson, G. Hong, D. Hill, G. Petersen, and F. Blattner.** 1983. Complete annotated lambda sequence, p. 519–576. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Davidson, A., and M. Gold.** 1987. A novel *in vitro* DNA packaging system demonstrating a direct role for the bacteriophage λ *FI* gene product. Virology **161:**305–315.
17. **Davidson, A. R., and M. Gold.** 1992. Mutations abolishing the endonuclease activity of bacteriophage λ terminase lie in two distinct regions of the *A* gene, one of which may encode a "leucine zipper" DNA-binding domain. Virology **189:**21–30.
18. **Feiss, M.** 1986. Terminase and the recognition, cutting and packaging of λ chromosomes. Trends Genet. **2:**100–104.
19. **Fowler, R. G., G. E. Degner, and E. C. Cox.** 1974. Mutational specificity of a conditional *Escherichia coli* mutator, *mutD5*. Mol. Gen. Genet. **133:**179–191.
20. **Frackman, S., D. A. Siegele, and M. Feiss.** 1984. A functional domain of bacteriophage λ terminase for prohead binding. J. Mol. Biol. **180:**283–300.
21. **Frackman, S., D. A. Siegele, and M. Feiss.** 1985. The terminase of bacteriophage λ: functional domains for *cosB* binding and multimer formation. J. Mol. Biol. **183:**225–238.
22. **Fry, D., S. Kuby, and A. Mildvan.** 1986. ATP-binding site of adenylate kinase: mechanistic implications of its homology with *ras*-encoded p21, F_1-ATPase, and other nucleotide-binding proteins. Proc. Natl. Acad. Sci. USA **83:**907–911.
23. **Gold, M., and A. Becker.** 1983. The bacteriophage λ terminase: partial purification and preliminary characterization of properties. J. Biol. Chem. **258:**14619–14625.
24. **Granston, A. E., D. M. Alessi, L. Eades, and D. Friedman.** 1988. A point mutation in the *Nu1* gene of bacteriophage λ facilitates phage growth in *Escherichia coli* with *himA* and *gyrB* mutations. Mol. Gen. Genet. **212:**149–156.
25. **Guo, P., C. Peterson, and D. Anderson.** 1987. Prohead and DNA-gp3-dependent ATPase activity of the DNA packaging protein gp16 of bacteriophage ϕ29. J. Mol. Biol. **197:**229–236.
26. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
27. **Higgins, R. R., and A. Becker.** 1994. Chromosome end formation in phage lambda, catalyzed by terminase, is controlled by two DNA elements of *cos*, *cosN*, and R3 and by ATP. EMBO J. **13:**6152–6161.
28. **Higgins, R. R., and A. Becker.** 1994. The lambda terminase enzyme measures the point of its endonucleolytic attack 47 ± 2 bp away from its site of specific DNA binding, the R site. EMBO J. **13:**6162–6171.
29. **Higgins, R., H. Lucko, and A. Becker.** 1988. Mechanism of *cos* DNA cleavage by bacteriophage λ terminase: multiple roles of ATP. Cell **54:**765–775.

29a. **Hwang, Y., and M. Feiss.** Unpublished data.

30. **Hwang, Y., C. E. Catalano, and M. Feiss.** 1996. Kinetic and mutational dissection of the two ATPase activities of terminase, the DNA packaging enzyme of bacteriophage λ. Biochemistry **35:**2796–2803.
31. **Hwang, Y., and M. Feiss.** 1996. Mutations affecting the high affinity ATPase center of gpA, the large subunit of bacteriophage λ terminase, inactivate the endonuclease activity of terminase. J. Mol. Biol. **261:**524–535.
32. **King, R. A., D. L. Anders, and G. E. Christie.** 1992. Site-directed mutagenesis of an amino acid residue in the bacteriophage P2 ogr protein implicated in interaction with *Escherichia coli* RNA polymerase. Mol. Microbiol. **6:**3313–3320.
33. **Kosturko, L. D., E. Daub, and H. Murialdo.** 1989. The interaction of *E. coli* integration host factor and lambda *cos* DNA: multiple complex formation and protein-induced bending. Nucleic Acids Res. **17:**317–334.
34. **Kuzminov, A., E. Schabtach, and F. W. Stahl.** 1994. Chi sites in combination with RecA protein increase the survival of linear DNA in *Escherichia coli* by inactivating the exoV activity of RecBCD nuclease. EMBO J. **13:**2764–2776.
35. **Kypr, J., and J. Mrazek.** 1986. Lambda phage protein Nu1 contains the conserved DNA binding fold of repressors. J. Mol. Biol. **91:**139–140.
36. **Mendelson, I., M. Gottesman, and A. B. Oppenheim.** 1991. HU and integration host factor function as auxiliary proteins in cleavage of phage lambda cohesive ends by terminase. J. Bacteriol. **173:**1670–1676.
37. **Pal, S. K., and D. K. Chattoraj.** 1988. P1 plasmid replication: initiator sequestration is inadequate to explain control by initiator-binding sites. J. Bacteriol. **170:**3554–3560.
38. **Rubinchik, S., W. Parris, and M. Gold.** 1994. The *in vitro* ATPases of bacteriophage λ terminase and its large subunit, gene product A. J. Biol. Chem. **269:**13586–13593.
39. **Rubinchik, S., W. Parris, and M. Gold.** 1994. The *in vitro* endonuclease activity of gene product A, the large subunit of the bacteriophage lambda terminase, and its relationship to the endonuclease activity of the holoenzyme. J. Biol. Chem. **269:**13575–13585.
40. **Shinder, G., and M. Gold.** 1988. The *Nu1* subunit of bacteriophage lambda terminase binds to specific sites in *cos* DNA. J. Virol. **62:**387–392.
41. **Sippy, J., and M. Feiss.** 1992. Analysis of a mutation affecting the specificity domain for prohead binding of the bacteriophage λ terminase. J. Bacteriol. **174:**850–856.
42. **Six, E., and C. A. C. Klug.** 1973. Bacteriophage P4: a satellite virus depending on a helper such as prophage P2. Virology **51:**327–344.
43. **Tomka, M. A., and C. E. Catalano.** 1993. Kinetic characterization of the ATPase activity of the DNA packaging enzyme from bacteriophage λ. Biochemistry **32:**11992–11997.
44. **Tomka, M. A., and C. E. Catalano.** 1993. Physical and kinetic characterization of the DNA packaging enzyme from bacteriophage λ. J. Biol. Chem. **268:**3056–3065.
45. **Walker, J., M. Saraste, M. Runswick, and N. Gay.** 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. **1:**945–951.
46. **Wiman, M., G. Bertani, B. Kelly, and I. Sasaki.** 1970. Genetic map of *E. coli* C. Mol. Gen. Genet. **107:**1–31.
47. **Wu, W.-F., S. Christiansen, and M. Feiss.** 1988. Domains for protein-protein interactions at the N and C termini of the large subunit of bacteriophage λ terminase. Genetics **119:**477–484.
48. **Xin, W., and M. Feiss.** 1988. The interaction of *Escherichia coli* integration host factor with the cohesive end sites of phages λ and 21. Nucleic Acids Res. **16:**2015–2030.
49. **Xin, W., and M. Feiss.** 1993. Function of IHF in λ DNA packaging. I. Identification of the strong binding site for integration host factor and the locus for intrinsic bending in *cosB*. J. Mol. Biol. **230:**492–504.
50. **Xin, W., Z.-H. Cai, and M. Feiss.** 1993. Function of IHF in λ DNA packaging. II. Effects of mutations altering the IHF binding site and the intrinsic bend in *cosB* on λ development. J. Mol. Biol. **230:**505–515.
51. **Xu, S.-Y., and M. Feiss.** 1991. Structure of the bacteriophage λ cohesive end site: genetic analysis of the site (*cosN*) at which nicks are introduced by terminase. J. Mol. Biol. **220:**281–292.
52. **Yeo, A., and M. Feiss.** 1995. Mutational analysis of the prohead binding domain of the large subunit of terminase, the bacteriophage λ DNA packaging enzyme. J. Mol. Biol. **245:**126–140.