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Analysis of a Mutation Affecting the Specificity Domain for Prohead Binding of the Bacteriophage λ Terminase

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Genetic studies have identified a specificity domain for prohead binding in the C-terminal 32 amino acids of gpA, the large subunit of bacteriophage λ terminase (S. Frackman, D. A. Siegele, and M. Feiss, J. Mol. Biol. 180:283–300, 1984). In the present work, an amber mutation, *A*am42, in the fifth-to-last codon of the *A* gene was found to be lethal in nonsuppressing hosts. The mutation, expected to generate gpA lacking the last five amino acids, caused the production of a terminase that cut *cos* efficiently both in vivo and in vitro but was defective in DNA packaging. λ *A*am42 lysates contained unused proheads, consistent with a defect in prohead binding. *A*am42 terminase was more strongly dependent than wild-type terminase on gpFI, the catalyst of prohead binding. Like wild-type terminase, *A*am42 terminase did not cut *cos* in vivo when prohead assembly was blocked by a mutation in one of the genes encoding the prohead.

The λ DNA packaging enzyme, terminase, is a heterooligomer of gpNu1, the 20.5-kDa product of the phage *Nu1* gene, and gpA, the 73-kDa product of the *A* gene (Fig. 1 shows an arrangement of terminase genes and terminase recognition sites). Terminase binds specifically to λ DNA at *cosB*, and the terminase-DNA complex binds a prohead, the empty protein shell into which DNA is to be packaged. Terminase also introduces staggered nicks at the cohesive nicking site, called *cosN*, in the concatemeric DNA to generate monomeric chromosomes possessing cohesive ends (reviewed in references 1 and 13). The *cosB* binding specificity is located in the amino terminus of gpNu1, at which a putative helix-turn-helix DNA binding motif is located. The *cosN* cutting activity of terminase is thought to be located in gpA (1). Both gpNu1 and gpA have ATPase activity, and it has been speculated that terminase acts as a translocase during DNA packaging (1). The carboxy terminus of gpA was implicated in prohead binding by Frackman et al. (17). Frackman et al. found that the terminases of phages λ and 21 were prohead specific and that the prohead binding specificity of 21 terminase switched from 21 proheads to λ proheads when the carboxy-terminal 32 amino acids of the large subunit of 21 terminase were replaced by the carboxy-terminal 32 amino acids of λ gpA. The binding of the prohead by the terminase-DNA complex is facilitated by morphogenetic protein gpFI (2, 11).

Frackman et al. (17) and Wu et al. (32) proposed that the carboxy-terminal 32 amino acids were a strap that specifically bound the prohead. As a first step towards understanding the terminase-prohead interaction, we have isolated and characterized a mutant of λ having a nonsense triplet in the fifth-to-last codon of the gpA open reading frame.

MATERIALS AND METHODS

Media. Tryptone broth (TB), top agar, and bottom agar were as described by Campbell (7), except that each was supplemented with 0.01 M $MgSO_4$. L broth (LB) and LB agar were as described by Bertani (3). When needed, kanamycin and ampicillin were added to LB and LB agar at 50 and 100 μg/ml, respectively. TB for infections was supplemented with 0.2% maltose.

Phages and bacterial strains. The phages and bacterial strains used are listed in Table 1.

Base pair coordinates. The λ numbering system is that of Daniels et al. (10), and restriction site coordinates are the 5′ base in each recognition site. The numbering of the pBR322 sequence begins within the unique *Eco*RI site, at the first T in the sequence 5′-GAATTC-3′, and proceeds into the tetracycline resistance determinant (6).

Sequencing and DNA manipulations. Sequences were determined with a sequencing kit from New England BioLabs, which uses the chain termination method of Sanger et al. (29). [α-^{35}S]dATP (Amersham) was used as the labeling nucleotide. Plasmid preparations were as described by Birnboim and Doly (4). Transformations, ligations, restriction digestions, and electrophoresis were done as described by Maniatis et al. (23).

Mutagenesis. The *Pst*I fragment of the λ *A* gene extending from bp 2556 to 2820 was cloned into the *Pst*I site of phage M13mp19. Oligonucleotide-directed mutagenesis was performed by the method of Kunkel (21). Isolates containing the *A*am42 mutation were identified as lacking the λ *Bsp*MII site at 2619, and the presence of the *A*am42 mutation was confirmed by sequencing.

Plasmid constructions. Wild-type terminase was expressed from pCM101Δ1, a deletion derivative of terminase expression plasmid pCM101 of Chow et al. (9). pCM101Δ1 was made as follows: a small segment of pCM101 extending from the *Ppu*MI site at bp 2815 in the λ *W* gene to the *Ppu*MI site at bp 1480 of pBR322 was deleted by digestion with *Ppu*MI followed by ligation.

Terminase-overproducing plasmid pCMA42 was constructed with pCM101 (9) and pBA42, a derivative of pBA4. Plasmid pBA4 is a derivative of pACYC184 (8) containing an insert of λ DNA extending from the *Hin*dIII site at 44141, through *cosN* and *cosB* (hereafter called *cos*), to the *Bgl*I site at 2660 in the λ *W* gene. For construction of pBA42, the *Pst*I fragment from the M13mp19 isolate containing the *A*am42 mutation was ligated into the single *Pst*I site located at 2556 in the λ DNA insert of pBA4, creating a tandem duplication of the carboxy terminus of the *A* gene and the beginning of the *W* gene. From pBA42, the fragment (containing the

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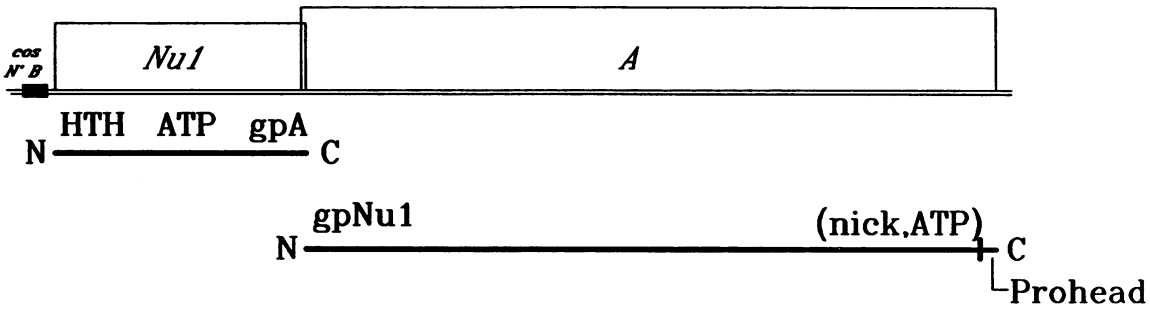

FIG. 1. Left end of the λ chromosome. *cosB* and *cosN* are the sites at which terminase binds and nicks λ DNA; *cosN'* indicates the portion of *cosN* at the left end of mature (cleaved) λ DNA. gpNu1 and gpA, the products of genes *Nu1* and *A*, are the small and large subunits of terminase, respectively. Designations: HTH, position of a putative helix-turn-helix (HTH) motif for *cosB* binding; ATP, ATP-reactive centers; gpA and gpNu1, domains for subunit-subunit interactions; nick, nicking domain thought to reside near the ATP-reactive center of gpA; Prohead, the specificity domain for prohead binding, at the carboxy terminase of gpA.

*A*am42 mutation) extending from the *Sph*I site at 2212 in gene *A* to the *Eco*0109 site at 2815 in gene *W* was substituted for the fragment of pCM101 extending from the *Sph*I site at 2212 in gene *A* to the *Ppu*MI site at 1480 of pBR322.

Plasmid pSX1, used in the *cos* cleavage assays, contains λ DNA from 47942, through *cos*, to 194 in vector pUC19 (34).

Plasmid pSF1, used as a probe in in vivo *cos* cleavage assays, is a derivative of pBR322 containing the λ DNA segment extending from the *Hin*dIII site at 44141, through *cos*, to the *Bam*HI site at 5505 (15).

TABLE 1. Phages and bacterial strains

Bacterial strain or phage	Relevant properties	Source or reference
Bacterial strain		
JM107	M13mp19 host	Our collection
CSH50	*dut*$^+$ *ung*$^+$	16
BW313	*dut ung*	16
MF1841	Cla *sup*$^+$	Our collection
MF611	*recA sup*$^+$	Our collection
C520	*supD*	Our collection
MS1285	*supP*	Our collection
C4514	*supE*	Our collection
C4518	*supF*	Our collection
FTP2028	*supT*	24
MF1913	*supT*	24
MF1914	*supT*	24
MF1915	*supT*	24
C4515	*supG*	Our collection
R594	*sup*$^+$	Our collection
C600	*supE*	Our collection
MF327	*supF*	Our collection
SKB178	*sup*$^+$	18
NS428	N100 (λ *A*am11 *b*2 *red*3 c1857 *S*am7)	31
Phages (λ)		
*A*am42 c1 857		This study
*W*am403 *red*3 c157		Our collection
*C*am20 c1857		Our collection
*A*am19 c1857		Our collection
*A*am11 c1857		Our collection
*FI*am785 c1857		Our collection
*A*am11 *FI* am785 c1857		Our collection
*FI*am785 c1857		This study
*E*am4 c1857		Our collection
*A*am42 *E*am4 c1857		This study
*A*am32 *red*3 *imm*21 cI		Our collection
P1:5R Kn^r c1857 *nin*5		28

Construction of λ *A*am42 c1857. pBA42, described above, was cut with *Eco*0109, yielding a fragment extending from the *Eco*0109 site at bp 48473, through *cos* and the terminase genes (with the *A*am42 mutation), to the *Eco*0109 site at bp 2815. The *A*am42 *Eco*0109 fragment of pBA42 was used to replace the *A*$^+$ *Eco*0109 segment of λ-P1:5R Kn^r c1857 *nin*5. λ-P1:5R c1857 *nin*5 was isolated by Sternberg and Austin (30), and it carries a 10-kbp segment of bacteriophage P1 DNA encoding functions for plasmid replication and partition. The λ-P1:5R c1857 *nin*5 prophage replicates as a single-copy plasmid with the P1 replicon at 32°C. At 42°C, however, the thermolabile (c1857) repressor is inactivated and the phage goes into lytic growth with the λ replication system. A derivative of λ-P1:5R c1857 *nin*5 containing a 1.3-kbp kanamycin resistance cassette was obtained from Dhruba Chattoraj (28). The genome size of λ-P1:5R Kn^r c1857 *nin*5, hereafter called λ-P1, is approximately 46.2 kbp. λ-P1 contains only two *Eco*0109 sites, at 48473 and 2815, so that one can conveniently replace the *cos* and terminase segment.

Complementations and burst studies. For complementations, *recA sup*$^+$ cells were grown in TB plus maltose to 10^8/ml. The cells were concentrated fivefold in 0.01 M $MgSO_4$, counted in a Petroff-Hauser chamber, and infected at a multiplicity of five phage per cell. After adsorption for 15 min at room temperature, the cells were treated with anti-λ serum (K, ~7 min^{-1}) for 7 min and diluted in TB to $<10^6$/ml. After incubation at 37°C for 70 min, the lysates were treated with chloroform and cell debris was spun down.

Burst size studies were done with heat-inducible lysogens of *sup*$^+$ cells which had been grown at 33°C to a density of 10^8/ml in TB. An aliquot was diluted and plated on tryptone agar plates for the production of single colonies at 33°C to determine a viable cell count. After induction for 15 min at 42°C, the cultures were examined for infectious centers and shaken for 70 min at 37°C. The lysates were treated with chloroform, clarified, and plated for phage yield on *supF* cells.

Terminase extracts. Terminase for the in vitro *cos* cleavage and packaging assays and for protein gel analysis was prepared from strain MF1841, carrying the terminase-overproducing plasmids pCM101Δ1 and pCMA42. The protocol used was that described by Murialdo et al. (27).

In vitro *cos* cleavage assay. The substrate for the in vitro *cos* cleavage assay was *cos*-containing plasmid pSX1, linearized by digestion with *Sca*I. Linearized pSX1, when cut at *cos* by terminase, yields 1,099- and 2,320-bp fragments. The assay conditions were those of Chow et al. (9), and crude terminase prepared as described above was used. Five microliters of linearized pSX1 DNA (0.25 μg) was mixed with 2.0 μl of DPB (6 mM Tris-HCl [pH 7.4], 18 mM $MgCl_2$, 30 mM spermidine, 60 mM putrescine)-ATP (85 μl of DPB and 15 μl of 100 mM ATP)–15 μl of DPA-MeSH (20 mM Tris-HCl [pH 8.0], 3 mM $MgCl_2$, 1 mM EDTA-KOH [pH 7.0], 7 mM β-mercaptoethanol)–5 μl of the crude terminase preparation. Terminase dilutions were made in uninduced cell extracts. After 30 min of incubation at room temperature, 3 μl of agarose gel loading buffer (0.25% bromophenol blue–40% [wt/vol] sucrose in water) was added. The samples were heated at 65°C for 5 min and run on an 0.8% agarose gel. The extent of *cos* cleavage was determined as described below for the in vivo *cos* cleavage assay.

Gel analysis of protein production. The procedure used for gel analysis of protein production was that outlined by Murialdo et al. (25), and crude terminase preparations were used. Cells were grown to 20 Klett units in LB-ampicillin. Aliquots of 1.0 ml were taken just prior to and immediately following thermoinduction at 45°C and at 15-min intervals thereafter, up to 60 min. Samples were kept on ice and centrifuged, and the pelleted cells were resuspended in loading buffer (0.25% bromophenol blue–0.25% xylene cyanol FF–40% [wt/vol] sucrose in water). After being treated with boiling water, they were loaded on a sodium dodecyl sulfate (SDS)-polyacrylamide gel (22) and subjected to electrophoresis overnight at 55 V. The gel was stained with Coomassie brilliant blue and photographed.

In vitro packaging assay. The in vitro packaging assay used was that described by Chow et al. (9), and the terminase extracts described above were used. A mixture of 30 μl of DPA-MeSH, 4 μl of λ *c*I857 *S*am7 DNA (0.4 μg; New England BioLabs), 4 μl of buffer (170 μl of DPB, 30 μl of 100 mM ATP, 2 μl of β-mercaptoethanol), 20 μl of a sonic extract of NS428 in DPA-MeSH, and 2 μl of crude terminase (diluted in DPA-MeSH) was incubated for 15 min at room temperature. The sonic extract of induced NS428, the source of proheads, was prepared as described by Blattner et al. (5). A 150-μl aliquot of a frozen-thawed lysate of NS428 was added, and incubation was continued for 60 min at room temperature. The frozen-thawed lysate of induced NS428, the source of tails, was prepared as described by Blattner et al. (5). Appropriate dilutions were plated on the *supF* strain MF327.

In vivo *cos* cleavage assay. The in vivo *cos* cleavage assay used was that described by Murialdo and Fife (26). Lysogens of SKB178 were grown in LB at 33°C to 10^8 cells per ml, induced for 15 min at 42°C, and shaken at 37°C for 20 min. Ten milliliters of the culture was added to a 30-ml Sorvall centrifuge tube containing 5 ml of frozen and crushed U1 buffer (50 mM NaCl, 0.1 mM EDTA, 40 mM Tris-HCl [pH 7.6]). The cells were pelleted by centrifugation at 4,300 × *g* and 4°C for 10 min. The pellet was resuspended in 500 μl of U1 buffer, treated with 10 μl of $CHCl_3$ at 37°C for 5 min, and extracted once with phenol-$CHCl_3$ (1:1) and three times with phenol. The aqueous phase was precipitated with NaCl-ethyl alcohol, and the resulting pellet was resuspended in 10 mM Tris-HCl (pH 7.5)–1 mM EDTA. DNA aliquots of from 1 to 4 μl were cut with *Bst*XI and subjected to electrophoresis on an 0.8% agarose gel. The DNA was vacuum transferred (Vacublot transfer system; American Bionetics) onto a nylon support membrane (GeneScreen Plus; New England Nuclear). Linearized, *cos*-containing plasmid pSF1 was prepared as a ^{32}P-labeled probe with a random primer DNA labeling kit (Boehringer Mannheim). The hybridization protocol was performed in accordance with the manufacturer's directions supplied with GeneScreen Plus. X-ray film exposures of the hybridized membrane were analyzed by densitometry on an LKB Ultroscan XL laser densitometer (Pharmacia LKB), with exposures which were in the linear response range. The peaks of interest were cut out and weighed on an analytical balance. The level of *cos* cleavage was calculated as follows: percent *cos* cleavage = [(weight of L end + weight of R end)/(weight of L end + weight of R end + weight of joint)] × 100, where L end and R end are the *Bst*XI fragments produced from the left and right ends, respectively, of mature λ DNA by *cos* cleavage and joint is the *cos*-containing *Bst*XI fragment of immature λ DNA that is the substrate for *cos* cleavage. DNA samples from induced lysogens of λ *A*am42 were found to contain L end but not R end, perhaps because of intracellular nuclease digestion of R-end DNA. For estimation of the amounts of *cos* cleavage for induced lysogens of λ *A*am42, the amount of R end produced by *cos* cleavage was calculated from the amount of L end produced by *cos* cleavage, with the assumption that equimolar amounts of L end and R end were produced by *cos* cleavage.

Phage-related structures. Concentrates of phage lysates were prepared by differential centrifugation as described by Xu and Feiss (33). Negative staining with phosphotungstate and electron microscopy were done as described by Huntley and Kemp (20).

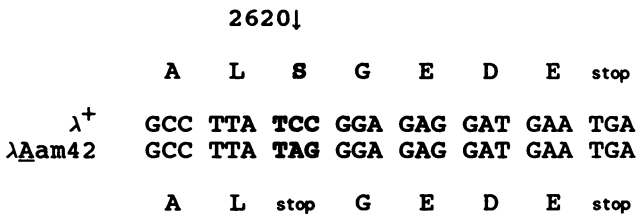

FIG. 2. Location of the *A*am42 mutation. Base pair 2620 is the center base pair of the serine codon, codon 637 of the *A* gene. The *A*am42 mutation converts the serine codon to an amber codon.

RESULTS

Construction and genetic analysis of λ *A*am42. Using oligonucleotide-directed mutagenesis, we generated an amber mutation at codon 637 of the λ *A* gene, the fifth codon from the carboxy terminus. The octadecanucleotide we used resulted in a transversion mutation at bp 2620 and 2621, changing the serine codon TCC to the amber codon TAG (Fig. 2). The λ *A*am42 phage was unable to form plaques on Cla (sup^+) cells, indicating that the last five amino acids of gpA are essential.

We considered the possibility that the *A*am42 mutation was lethal in sup^+ cells because of polarity on downstream genes. Accordingly, we examined whether λ *A*am42 could complement λ *W*am and λ *C*am. We found that downstream genes were expressed normally, as λ *A*am42 provided gpW and gpC as well as a control *A*am phage, λ *A*am32 (Table 2, infections 6, 7, and 8). Coinfection with λ^+ and λ *A*am42 resulted in good yields of both phages, so the *A*am42 mutation was recessive (Table 2, infections 9 and 10).

To determine whether amino acids other than serine would allow functional terminase to be produced, we plated

TABLE 2. Complementation of *A*am42

Infection[a]	Infecting phage(s)	Yield[b]
1	λ$^+$	67.5
2	λ *W*am403[c]	$<1.0 \times 10^{-3}$
3	λ *C*am20[c]	$<1.0 \times 10^{-3}$
4	λ *A*am42[d]	3.9×10^{-3}
5	λ *A*am32[e]	$<1.0 \times 10^{-3}$
6	λ *W*am403 + λ *A*am32	13.3
7	λ *W*am403 + λ *A*am42	23.3
8	λ *C*am20 + *A*am42	21.6
9	λ$^+$ + λ *A*am32	35.8
10	λ$^+$ + λ *A*am42	26.2

[a] Performed as described in Materials and Methods.
[b] Number of phage per infected cell; average of two experiments.
[c] *c*I857 *red*3.
[d] *c*I857.
[e] *imm*21 *c*I *red*3.

λ *A*am42 on cells with suppressors that insert different amino acids. All of the suppressors tested were permissive; they were (inserted amino acid in parentheses) *supD* (S), *supE* (Q), *supF* (Y), *supP* (L), *supT* (G), *supU* (W), and *supG* (K). The variety of amino acids that allowed the production of functional terminase when inserted at position 637 indicated that serine is not crucial for the terminase-prohead interaction.

The *A*am mutation was leaky and λ *A*am42 required *FI*. Burst size measurements were made with Cla (*sup*$^+$) cells lysogenized by λ *A*am42 *c*I857, λ *A*am19 *c*I857, and λ *c*I857 (Table 3). The infectious center level for λ *A*am42 was only 5- to 10-fold lower than that for the *A*$^+$ phage (λ *c*I857), whereas for another *A*am phage, λ *A*am19, the level was 10^{-6}- to 10^{-7}-fold the level for the *A*$^+$ phage. Note that the burst size of λ$^+$ was much higher in this experiment than in the experiment shown in Table 2. One reason for the difference is that a *recA* host was used in the experiment in Table 2, whereas in the experiment in Table 3 (and that in Table 4), a *rec*$^+$ host was used. Zissler et al. (35) found the burst size of λ$^+$ to be twofold lower in an infection of *recA* cells (as in our experiment in Table 2) than with the induction of a *recA*$^+$ lysogen (as in our experiments in Tables 3 and 4). An additional factor is that the λ$^+$ phage used in the experiment in Table 2 was *c*I$^+$, a characteristic which we have found to reduce the burst size in similar infections by a factor of 2, in comparison with that in infections with phages that are *c*I (14). The yields of phage per induced cell also showed that the *A*am42 mutation was leaky. Only one phage was produced for every 10^5 induced λ *A*am19 lysogens, whereas λ *A*am42 lysogens produced one phage for every 10 induced cells. The leakiness of the *A*am42 mutation was also found in in vitro packaging studies (see below).

TABLE 3. Burst size study of λ *A*am42

Expt	Prophage[a]	Infectious center(s)[b]	Yield[c]
1	λ$^+$	1.2	434
	λ *A*am19	3.5×10^{-7}	1.4×10^{-5}
	λ *A*am42	0.27	0.09
2	λ$^+$	0.97	374
	λ *A*am19	1.09×10^{-6}	1.5×10^{-5}
	λ *A*am42	0.12	0.10

[a] *c*I857 in Cla (*sup*$^+$) cells.
[b] Number of cells yielding viable phage/number of induced cells.
[c] Number of phage per induced cell.

TABLE 4. *FI* requirement of λ *A*am42

Prophage[a]	Infectious center(s)[b]	Yield[c]
λ$^+$	1.21	446
λ *A*am11	2.84×10^{-4}	8.4×10^{-5}
λ *A*am42	0.16	0.16
λ *FI*am 785	0.42	1.7
λ *A*am11 *FI*am785	5.07×10^{-6}	2.0×10^{-7}
λ *A*am42 *FI*am785 Iso1[d]	2.89×10^{-6}	2.8×10^{-6}
λ *A*am42 *FI*am785 Iso2[d]	3.76×10^{-6}	1.0×10^{-5}

[a] *c*I857 in R594 (*sup*$^+$) cells.
[b] See Table 3, footnote *b*.
[c] See Table 3, footnote *c*.
[d] Independent isolate.

The level of leakiness of *A*am42 in the burst studies was similar to that exhibited by *FI* amber mutants (27). The *FI* gene product acts as a catalyst in complex II formation, stimulating the binding of a prohead by the DNA-terminase complex (2, 11). Since the level of leakiness observed for λ *A*am42 was similar to that observed for λ *FI* strains, we wondered whether the *A*am42 mutation simply abolished the ability of terminase to respond to gpFI. Accordingly, we examined whether λ *A*am42 required *FI*. Burst studies were again conducted with lysogens of R594 (*sup*$^+$) (Table 4). As expected, λ *A*am42 and the *FI* amber mutants produced relatively large numbers of infectious centers (Table 4, rows 3 and 4). In contrast, the double mutant λ *A*am42 *FI*am785 produced very few infectious centers and a very small burst size (Table 4, row 6). It is clear that λ *A*am42 is strongly dependent on the *FI* gene product.

In vitro studies of *cos* cleavage and DNA packaging by *A*am42 terminase. *A*am42 terminase was used in an in vitro *cos* cleavage assay, in which linearized plasmid DNA containing *cos* was treated with terminase and the fragments were resolved on an agarose gel. Terminase preparations were extracts of C1a cells carrying isogenic terminase expression plasmids encoding wild-type or *A*am42 terminase. SDS-polyacrylamide gel analysis showed that the two terminase preparations contained identical amounts of terminase (data not shown). The *cos* cleavage assays showed that *A*am42 terminase cut *cos* as well as wild-type terminase did (Fig. 3).

In the in vitro DNA packaging assay, DNA and terminase are mixed with a sonic extract containing phage proheads and host factors necessary for packaging. After a brief incubation period, phage tails are added to make complete phage particles. The phage mixture is diluted and plated on cells for the production of PFU. With this assay, λ DNA was packaged with wild-type and *A*am42 terminases. *A*am42 terminase packaged λ DNA about 25-fold less efficiently than did wild-type terminase (Table 5).

In vivo *cos* cleavage assay. In vitro, *A*am42 terminase cut as well as wild-type terminase did, yet it had a defect in packaging (25-fold). We wanted to determine whether *A*am42 terminase was able to cleave *cos* efficiently in vivo. In addition, we examined whether *A*am42 terminase was subject to maturation inhibition, i.e., inhibition of *cos* cleavage in the absence of functional proheads. Maturation inhibition is observed when prohead formation is blocked by an amber mutation in one of the prohead genes (26). We examined maturation inhibition of *A*am42 terminase by examining *cos* cutting during the development of λ *A*am42

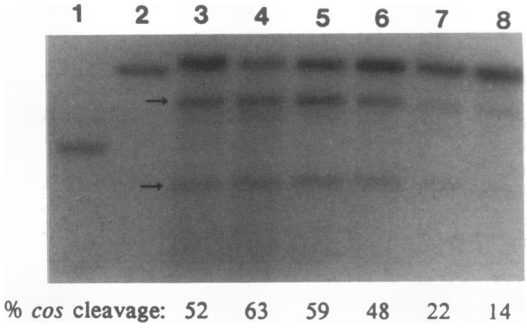

FIG. 3. In vitro *cos* cleavage assay. *cos* cleavage products (1,099 and 2,320 bp) of substrate pSX1 are marked by arrows. Lanes: 1, 1,635-bp size standard; 2, linearized pSX1, no terminase; 3 and 4, undiluted wild-type and *A*am42 terminases; 5 and 6; 10-fold-diluted wild-type and *A*am42 terminases; 7 and 8, 100-fold-diluted wild-type and *A*am42 terminases. The slightly slower mobility of the linearized plasmid in lanes 3 through 8, relative to the mobility in lane 2, is ascribed to the presence of a cell extract in lanes 3 through 8. Percent *cos* cleavage values are given below the autoradiogram.

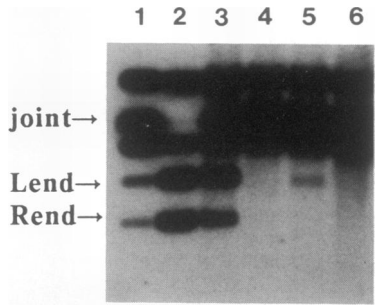

FIG. 4. In vivo *cos* cleavage assay. Lanes 1 and 2 are control lanes of λ *c*I857 *S*am7 DNA cut with *Bst*XI. The DNA in lane 1 was unheated, so most of the L-end and R-end fragments were annealed and migrated as the joint fragment. The DNA in lane 2 was heated at 65°C for 5 min prior to being loaded on the agarose gel to melt hydrogen-bonded cohesive ends; hence, most of the joint fragment was disrupted to form L-end and R-end fragments. Lanes 3 to 6 are *Bst*XI digests of prophage DNA from SKB178 cells: lane 3, λ^+; lane 4, λ *E*am4; lane 5, λ *A*am42; lane 6, λ *A*am42 *E*am4. The DNA in lanes 3 to 6 was heated at 65°C for 5 min prior to being loaded on the agarose gel to melt hydrogen-bonded cohesive ends. Cleavage of the fragment, labeled joint, by terminase generated L-end and R-end fragments, which corresponded to the *Bst*XI fragments from the left and right ends of mature λ DNA, respectively. Bands not marked with arrows are those which flanked the *cos* fragments and contained sequences homologous to the probe.

*E*am4. Lysogens of SKB178, a *sup*$^+$ host, were prepared for λ *c*I857, λ *c*I857 *E*am4, λ *c*I857 *A*am42, and λ *c*I857 *A*am42 *E*am4. For examination of *cos* cleavage in vivo, DNA was isolated, cut, and probed as described in Materials and Methods. The *Bst*XI fragment containing uncut *cos* is 4.9 kbp in length and is called the joint fragment. Cleavage of the joint fragment by terminase generates a 2.1-kbp piece (called R end) from the right (*Rz* gene) end of the λ chromosome and a 2.8-kbp piece (called L end) from the left (*Nu*1 gene) end. *cos* cutting was observed for λ A^+ (64%) and λ *A*am42 (12%) but not for λ A^+ *E*am4 (<2%) or λ *A*am42 *E*am4 (<2%) (Fig. 4). The results with the *E*am phages indicated that *A*am42 terminase, like wild-type terminase, was subject to maturation inhibition. There was a clear difference between the levels of *cos* cleavage during infections with λ A^+ and λ *A*am42, but the difference was much too small to account for the 4,000-fold difference in phage production by the two phages. We conclude that the primary defect in λ *A*am42 terminase is not in the ability to cut *cos*.

Head structures in λ *A*am42 lysates. Packaging of DNA into the λ prohead is accompanied by an expansion of the prohead. Prohead expansion occurs when the prohead has been packed with a length of DNA that is between 11 and 45% the λ^+ chromosome length of 48.5 kbp (19). Prohead expansion, therefore, is an indication that some DNA packaging has occurred. Examination of structures produced by λ A^+ and λ *A*am42 showed that prohead expansion did not occur during infection with λ *A*am42 (Table 6).

DISCUSSION

The *A*am42 mutation, generated by oligonucleotide-directed mutagenesis, creates an amber mutation at codon 637 of the *A* gene. Amino acid 637 of gpA need not be the serine present in wild-type gpA, because the *A*am42 mutation is suppressed by amber suppressors that insert seven different amino acids. In *sup*$^+$ hosts, in which the *A*am42 mutation is lethal, λ *A*am42 is expected to produce gpA lacking the last five amino acids, although we cannot exclude the possibility that deletion of the last five amino acids renders the C terminus of gpA susceptible to protease digestion. Any protease nibbling of the C terminus of *A*am42 gpA, if it occurs, must not cause the loss of many additional amino acids, because the *A*am42 mutation causes no obvious change in the mobility of gpA in SDS-polyacrylamide gel electrophoresis. Poor expression of adjacent downstream genes is not the cause of the lethality of the *A*am42 mutation, because λ *A*am42 supplies functional gpW and gpC in complementation tests. The *A*am42 mutation is leaky, however, in that the burst size of λ *A*am42, while too small for plaque formation, is 10^4-fold greater than that of λ *A*am19.

Cleavage of *cos* by *A*am42 terminase. In vitro, *A*am42 terminase is as efficient as wild-type terminase at *cos* cleavage. In vivo, the level of *cos* cleavage for λ *A*am42 is ca. fivefold lower than that for λ A^+. The in vivo *cos* cleavage

TABLE 5. In vitro packaging with mutant terminase

Reaction	Terminase[a]	Yield[b]
1	λ^+ (1:10)	2.19×10^7
2	λ *A*am42 (1:10)	9.42×10^5
3	λ^+ (1:30)	8.60×10^6
4	λ *A*am42 (1:30)	2.85×10^5

[a] From overproducing plasmids pCMA42 (*A*am42) and pCM101Δ1 (A^+).
[b] PFU/μg of DNA; average of three experiments. Yield ratios (λ *A*am42/λ^+) were 0.042 for reactions 1 and 2 and 0.033 for reactions 3 and 4.

TABLE 6. Morphogenesis by λ *A*am42

Phage	No. of the following phage-related structures in lysates:	
	Phages	Proheads
λ^+	46	88
λ *A*am19	0	88
λ *A*am42	0	470
λ *Fl*am785	0	157

comparison is complicated by the fact that DNA packaging occurs in the λ A^+ infection but not in the λ *A*am42 infection. In the λ A^+ infection, because *cos* cleavage is accompanied by DNA packaging, much of the cut DNA is sequestered in phage heads, where it is protected from degradation. In contrast, in the λ *A*am42 infection, the cut DNA is not packaged and may be subject to exonucleolytic digestion. A second complication results from processive packaging of λ chromosomes. In λ infections, chromosomes are packaged from concatameric DNA in a series estimated to be two or three chromosomes in length (12, 16). The first *cos* cleavage in the series is an initiating cut, whereas the downstream cleavages are a consequence of processive packaging. In the case of infection with λ *A*am42, the downstream cuts will not occur if packaging of the initial chromosome in the series has not taken place, so a reduced level of *cos* cleavage is expected in λ *A*am42-infected cells even if the terminase is fully active at *cos* cleavage.

While these complications make direct comparison between the levels of *cos* cleavage by λ A^+ and λ *A*am42 difficult, the level of *cos* cleavage by λ *A*am42 is sufficiently high to indicate that the primary defect in *A*am42 terminase is not in *cos* cleavage. Because there is no obvious defect in *cos* cleavage, it is unlikely that *A*am42 terminase is defective in binding to *cosB* and nicking of *cosN*.

DNA packaging by *A*am42 terminase. In vitro, *A*am42 terminase is less efficient at DNA packaging than wild-type terminase. It is not clear why in vitro DNA packaging with *A*am42 terminase is only reduced 25-fold, whereas λ *A*am42 has a phage yield that is 4,000-fold lower than that of λ^+. Previous studies have noted differences between in vitro and in vivo DNA packaging, such as the observation that gpFI is not needed for DNA packaging under typical conditions (2, 11). To demonstrate a strong dependence on gpFI in an in vitro packaging system, Davidson and Gold (11) diluted both the terminase and prohead extracts. The DNA packaging defect in *A*am42 terminase would perhaps be greater under in vitro conditions different from those used here. Because *A*am42 terminase has no demonstrable defect in interactions with *cos*, we conclude that the in vitro packaging defect occurs at a stage after the formation of complex I. The accumulation of proheads in lysates of λ *A*am42 suggests that the defect in packaging occurs at a stage prior to prohead expansion, indicating that if packaging of DNA into proheads is initiated during an infection with λ *A*am42, the process is aborted before prohead expansion. Because we have not shown directly that *A*am42 terminase is defective in complex II formation, we have not excluded the possibility that *A*am42 terminase is active in complex II formation but defective in a subsequent stage, such as DNA translocation.

Regulation of *A*am42 terminase action. The *FI* gene product has been shown to stimulate the binding of proheads by complex I, the complex of terminase and λ DNA (2, 11). We found that *A*am42 terminase requires gpFI and in fact is more dependent on gpFI than wild-type terminase is. A mutation in *FI* reduces the yield of λ^+ by a factor of 250, whereas an *FI* mutation reduces the yield of λ *A*am42 by a factor of more than 1,000 (Table 4). *A*am42 terminase clearly retains the ability to respond to gpFI.

cos cleavage in vivo is subject to maturation inhibition, the blocking of *cos* cleavage that is found when prohead assembly is prevented by an amber mutation in one of the genes encoding the prohead (26). The molecular basis of maturation inhibition is obscure, but it is clear that *A*am42 terminase is subject to this control for *cos* cleavage activity (Table 6).

Strap model for the prohead binding specificity domain. We previously proposed that the C-terminal 32 amino acids of terminase acted as a strap or tether by which terminase in complex I bound the prohead (17, 33). In the present work, we show that an amber mutation in the fifth-to-last amino acid of the *A* gene generates a terminase that retains the ability to cut *cos* but has a defect in DNA packaging. The results are consistent with the model which predicts that the *A*am42 mutation should cause a defect in the formation of complex II. Although we have not demonstrated directly a defect in the formation of complex II, our results indicate that the *A*am42 defect occurs at a step after the formation of complex I and prior to DNA packaging to an extent sufficient for prohead expansion, consistent with a defect in the formation of complex II.

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