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Isolation and Characterization of Mutations in the Bacteriophage λ Terminase Genes

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The terminase enzyme of bacteriophage λ is a hetero-oligomeric protein which catalyzes the site-specific endonucleolytic cleavage of λ DNA and its packaging into phage proheads; it is composed of the products of the λ *Nu1* and *A* genes. We have developed a simple method to select mutations in the terminase genes carried on a high-copy-number plasmid, based on the ability of wild-type terminase to kill *recA* strains of *Escherichia coli*. Sixty-three different spontaneous mutations and 13 linker insertion mutations were isolated by this method and analyzed. Extracts of cells transformed by mutant plasmids displayed variable degrees of reduction in the activity of one or both terminase subunits as assayed by in vitro λ DNA packaging. A method of genetically mapping plasmid-borne mutations in the *A* gene by measuring their ability to rescue various λ *A*am phages showed that the *A* mutations were fairly evenly distributed across the gene. Mutant *A* genes were also subcloned into overproducing plasmid constructs, and it was determined that more than half of them directed the synthesis of normal amounts of full-length *A* protein. Three of the *A* gene mutants displayed dramatically reduced in vitro packaging activity only when immature (uncut) λ DNA was used as the substrate; therefore, these mutations may lie in the endonuclease domain of terminase. Interestingly, the putative endonuclease mutations mapped in two distinct locations in the *A* gene separated by at least 400 bp.

The *Nu1* and *A* gene products (gpNu1 and gpA, respectively) of bacteriophage λ form a multifunctional enzyme, known as terminase, which plays a central role in the assembly of the mature virion (for reviews, see references 4, 10, and 11). During late stages of the λ lytic life cycle, the phage DNA is replicated into tandem concatemers consisting of many genomes joined end to end in a linear array. At the same time, empty phage heads, or proheads, are formed by a separate pathway. Terminase binds specifically to λ DNA and proheads and then packages the DNA into the prohead. Terminase also has endonuclease activity and cleaves the phage DNA at a specific site known as *cos*, so that single genome lengths are packaged into each phage head. The concatemeric form of λ DNA is referred to as immature, whereas the cleaved form which is inside the phage head is called mature. The terminase-mediated cleavage at *cos* produces 12-bp single-stranded DNA cohesive ends which are complementary and can anneal when the phage DNA is injected into a cell upon infection. In vitro studies have shown that terminase is an ATPase; however, in vitro *cos* cleavage requires only the presence of ATP, not its hydrolysis (15). ATP hydrolysis has been shown to be essential for the terminase-mediated melting of the cohesive ends produced in the cleavage reaction (18) and is also presumed to drive the packaging reaction.

Within the structures of gpNu1 (21 kDa) and gpA (74 kDa) there must be domains responsible for the many different interactions which subserve the overall packaging process. Various investigations have shed light on the location of some of these domains. The study of viable chimeras of λ and its close relative, phage 21, has demonstrated that the amino terminus of gpNu1 is involved in DNA recognition; the carboxy terminus of gpNu1 interacts specifically with the amino terminus of gpA, and the 38 carboxy-terminal amino acid residues of gpA are involved in the recognition and binding of proheads (13, 14, 41). In vitro studies have confirmed that gpNu1 does bind to *cos* DNA at specific sites (36). In addition, highly purified gpNu1 has been shown to possess ATPase activity (3, 32). Examination of the gpNu1 amino acid sequence as predicted from the DNA sequence has revealed the presence of a consensus helix-turn-helix DNA-binding fold from residues 3 to 24 (10, 21) and a consensus ATP-reactive center at residues 29 to 49 and 103 to 130 (3). A metal-binding site starting at residue 232 and an A-type ATP-binding domain from residues 484 to 505 have been predicted in the *A* gene (6, 17). So far, however, there are no data pertaining to the locations of the terminase domains responsible for the endonucleolytic and packaging activities.

To gain a clearer understanding of the location of terminase domains and how they interact in the holoenzyme, we have set out to isolate a large number of point mutations and linker insertion mutations in the *Nu1* and *A* genes and to characterize the in vitro activities of the mutant proteins.

MATERIALS AND METHODS

Media and chemicals. The medium used for cell growth was Luria broth (LB). Nutrient agar plates were used for bacterial colony formation and phage plaque assays (29). Ampicillin was added to nutrient agar plates and LB at a concentration of 40 μg/ml.

Restriction enzymes were obtained from Boehringer Mannheim, Bethesda Research Laboratories, New England BioLabs, and Pharmacia P-L Biochemicals. The 8-mer *Xho*I linker and *Bam*HI-*Sma*I adaptor were obtained from Pharmacia P-L Biochemicals. Other linkers used were synthesized in an Applied Biosystems 3890 DNA synthesizer.

Bacterial and bacteriophage strains. Bacterial and bacteriophage strains used in this study are listed in Table 1. All bacteria are derivatives of *Escherichia coli* K-12.

DNA techniques. DNA isolation, digestion, ligation, electrophoresis, bacterial transformation, and extraction of ma-

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TABLE 1. Bacterial strains and bacteriophages

Strain or phage	Relevant feature(s)	Reference
E. coli K-12		
594	Sup0	40
TC600	*supE*	30
QD5003	*supF*	43
N205	*recA*	39
AZ1544	N205 *recA*$^+$	26
OR1265	Constitutively expresses λ *c*I857 gene from a cryptic prophage, used as a host for pCM2 and pCM230	34
JM101	Host for pTZ18R and its derivatives	42
NS428	Sup0 *recA* (λ*A*am11 *b2 red*3 *c*I857 *S*am7)	38
Bacteriophages		
λ *A*am11 *imm*^{21} *c*I*ts*		8
λ *A*am19 *imm*^{434}		8
λ *A*am32 *imm*^{434}		8
λ *A*am36 *imm*^{434}		8
λ *A*am854 *imm*^{434}		31
λ *A*ama1 *imm*^{434}		19
λ *A*ama2 *imm*^{434}		19
λ *A*ama3 *imm*^{434}		19
λ *A*amps3 *imm*^{434}	Also known as *A*ama19	5

ture λ DNA from plate lysates were performed by standard methods (23).

Plasmids. The plasmid used to express terminase was pFM123 (see Fig. 1) (26). The *Nu1* and *A* genes on this plasmid are transcribed from the λ p_R and p_L promoters in tandem configuration. This promoter is repressed at 30°C by the *c*I857 temperature-sensitive λ repressor which the plasmid expresses constitutively. At 42°C the *c*I857 repressor is inactivated and transcription can proceed from the p_R and p_L promoters. Therefore, cells transformed by pFM123 express terminase at 42°C, but not at 30°C. pCM230 is a 21.7-kbp plasmid containing the whole morphogenetic region of λ except for a 1.8-kbp deletion within the *Nu1* and *A* genes. The deletion extends from λ bp 654 to 2088, so that the last 80 bp of the *Nu1* gene and the first 1,300 bp of the *A* gene are removed. pCM230 extracts have no gpNu1 or gpA activity. pCM230 also contains the *c*I857 gene and a promoter arrangement identical to pFM123; thus, cells transformed by this plasmid express the morphogenetic genes at temperatures over 40°C (9). pCM2 is a plasmid which can direct the synthesis of high levels of gpA (27). pTZ18R (Pharmacia) was used as a vector for subcloning and sequencing fragments from mutant plasmids. It contains the *lac* operon region and multiple-cloning site from pUC18 (42) and also has an f1 phage origin of replication so that single-stranded DNA can be made.

A cloning vector, called pAD15, was used for constructing *A* gene deletion plasmids from linker insertion mutations. This plasmid is a derivative of pMOB48 which is a 9.4-kbp plasmid containing the chloramphenicol resistance gene, an R1 origin of replication, and the *lac* and multiple-cloning-site regions from M13mp7 (7). pAD15 is identical to pMOB48, except that an *Xho*I linker was inserted at a *Sma*I site, creating a unique *Xho*I site positioned approximately 1 kbp from a unique *Bam*HI site which lies in the M13 multiple cloning site region. Deletion plasmids were constructed by cloning *Bam*HI-*Xho*I fragments from plasmids containing *Xho*I linker insertions (see Table 2) in the *A* gene into pAD15 cut with *Bam*HI and *Xho*I. The *Bam*HI site in pFM123 lies before the start of the *Nu1* gene (Fig. 1). Thus, the plasmids constructed contained the whole *Nu1* gene and the 5′ portion of the *A* gene up to the point where the linker was inserted.

gpA-overproducing plasmids containing *A* gene mutations were constructed by replacing the 2.0-kbp *Eco*RI-*Hpa*I fragment of pFM123 containing the promoter region, *Nu1* gene, and the first 24 bp of the *A* gene with a 1.9-kbp *Eco*RI-*Hpa*I fragment from pCM2. The pCM2 fragment contains the λ p_L promoter and the first 24 bp of the *A* gene preceded by the strong λ *c*II gene translation start site.

DNA sequencing of amber mutations. Sequencing reactions were carried out by using the T7 DNA Polymerase Sequencing Kit (Pharmacia). All sequencing was primed from the M13 universal primer included in this kit. Sequencing was performed on double-stranded plasmid DNA minipreparations of the constructions described in Table 4. Since the approximate positions of the amber mutations were already known from genetic analyses, it was possible to construct the plasmids in such a way that the amber mutation to be sequenced lay within 250 bp of the hybridization site of the universal primer. In this way, the mutations could be sequenced without further subcloning.

Growth of cells and preparation of extracts for in vitro packaging. Extracts of cells transformed by pCM230 or pCM2 and NS428 were prepared from 500-ml cultures as previously described (27, 28). Extracts of N205 cells containing plasmids with a mutant terminase gene were prepared by growing a 100-ml culture started with 1 ml of a fresh overnight culture for 6 h at 42°C. The culture was then centrifuged at 13,000 × *g* for 10 min. The pellet was resuspended in 3 ml of buffer A (20 mM Tris-HCl [pH 8.0], 1 mM Na_2EDTA, 3 mM $MgCl_2$, 5 mM β-mercaptoethanol) (2) and sonicated on ice with 8 to 10 15-s pulses. Cellular debris were removed by centrifugation at 2,600 × *g* for 5 min.

In vitro packaging assays. All assays contained 30 μl of buffer A, 4 μl of buffer B (6 mM Tris-HCl [pH 7.4], 15 mM ATP, 18 mM $MgCl_2$, 60 mM spermidine, 30 mM β-mercaptoethanol) (2), and either 4 μl of a 250-mg/ml solution of mature λ *c*I857 *S*am7 DNA (prepared from phage purified on CsCl gradients as described in reference 1) or 10 μl of a 1-mg/ml solution of immature λ *A*am11 *c*I857 *S*am7 DNA (prepared as described by McClure et al. [24]). To assay gpNu1 activity, the reaction mixtures also included 10 μl of an extract of OR1265 cells transformed by pCM230 and 10 μl of a 10-fold-diluted extract of OR1265 cells transformed by pCM2. The pCM230 extract served as the source of all the λ morphogenetic components except gpNu1 and gpA, while the pCM2 extract provided an excess of wild-type gpA. gpA activity was assayed by adding 10 μl of the extract to be tested to 20 μl of an extract from induced NS428 cells. The NS428 extract provided all the λ morphogenetic components and gpNu1. Unless otherwise indicated, all reactions were carried out at room temperature (22°C) and were stopped after 30 min by the addition of 5 μl of a 4-mg/ml pancreatic DNase 1 (Cooper Biomedicals) solution in 0.01 M HCl. The reactions were set up so that all the components required for packaging were in excess except the terminase subunit being assayed; thus, the number of phage produced in the reaction depended on the level of gpNu1 or gpA in the extract being tested. Strain QD5003 was used for determination of the titer of the phage produced in the reactions. The average activity obtained with wild-type extracts was 2.0×10^7 PFU/ml of

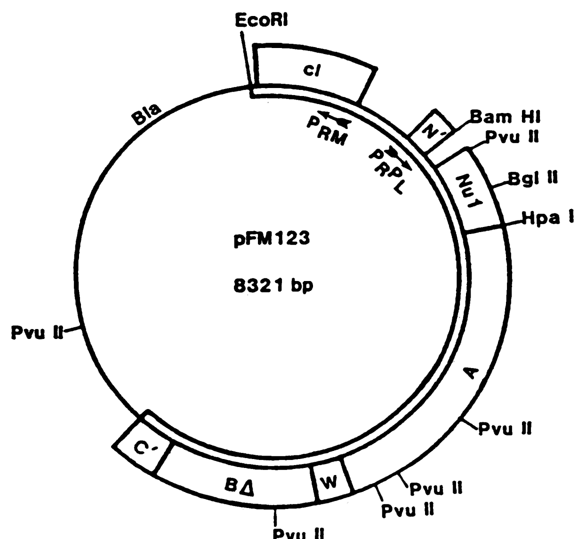

FIG. 1. Map of pFM123. The region of the plasmid containing phage DNA is shown as a double line. DNA of pBR322 origin is shown as a single line. λ genes are shown as boxes, and their names are indicated inside the boxes. Truncated genes are symbolized by a superscript prime after their name. The delta beside the *B* gene indicates that it contains an internal deletion. Bla stands for the β-lactamase gene. Arrows indicate the positions and directions of promoters relevant to this work. The positions of some of the restriction enzyme sites used in this work are indicated.

reaction mix in the gpNu1 assay and 3.1×10^8 PFU/ml of reaction mix in the gpA assay. The standard error associated with these averages is approximately 35%.

Measurement of gpA mutant protein production. OR1265, a strain which constitutively expresses temperature-sensitive λ *c*I repressor protein, was transformed by gpA-overproducing plasmids containing mutant *A* genes (see above). These cells were grown in 2.5-ml cultures at 30°C for 2.5 h, and then gpA expression was induced by growth at 42°C for 15 min. After induction, the cells were grown for an additional 30 min at 39°C. Then 1.2 ml of culture was spun down and resuspended in 100 μl of sample buffer, and 30 μl of this mixture was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 10% acrylamide) as previously described (22, 27).

Marker rescue experiments. Fresh overnight cultures of plasmid-transformed cells were diluted fourfold in λ diluent (10 mM Tris-HCl [pH 7.5], 10 mM $MgSO_4$, 10 mg of gelatin per ml), and 0.2 ml of the diluted cell suspension was added to 0.1 ml of phage solution (usually diluted in LB to 5×10^7 PFU/ml). This mixture was incubated at 30°C for 30 min and then poured with 2.5 ml of top agar onto 35-ml nutrient agar plates. The plates were incubated overnight at 30°C. Since the mutations to be mapped were carried on plasmids expressing λ repressor, heteroimmune phages were used; thus, all the phages used were imm^{434}, except for λ *A*am11, which was imm^{21}. The results shown for spontaneous mutants are based on at least two repetitions, whereas those shown for the linker insertions and amber mutation plasmids are based on at least three repetitions. The average standard error in these experiments was approximately 20%.

To map *A*am mutations, we plated λ *A*am derivatives by standard techniques on lawns of 594 (Sup0) cells carrying various *A* gene deletion plasmids. Plates were incubated overnight at 37°C. λ *A*am phage generally plated approximately 10^6-fold less efficiently on 594 cells than on suppressor strains.

RESULTS

Isolation and mapping of linker insertion mutations in the terminase genes. Terminase, when expressed from a high-copy-number plasmid, can kill certain *E. coli* strains (e.g., *recA* strains) that are deficient in DNA repair and recombination (26). This lethality is most probably due to the inability of these cells to repair damage that terminase inflicts by cleaving cryptic *cos* sites in the *E. coli* genome. This terminase-induced lethality was used as a means to select for mutations in the terminase genes carried on a plasmid.

A terminase-expressing plasmid called pFM123 (Fig. 1) was mutagenized by partial digestion with blunt-cutting restriction endonucleases and ligation to *Xho*I linkers. Plasmids bearing insertions in the terminase genes were selected by transforming the ligation mixtures into N205, an *E. coli recA* strain, and picking colonies that survived at 42°C on plates containing ampicillin. Under these conditions, N205 cells carrying wild-type pFM123 are killed because terminase expression is induced at the high temperature. By using this method, linker insertion mutations at 11 positions in the *Nu1* and *A* genes of pFM123 were isolated, as shown in Table 2. Two other linker insertion mutations, LM116 and LM117, were made by simply ligating linkers into unique restriction enzyme sites. The positions of the linkers within the terminase genes were mapped by digestion with various restriction enzymes and analysis of the fragment sizes produced. It should be noted that only pLM110, pLM112, pLM114, and pLM119 contain in-frame insertions (i.e., a 12-bp linker was inserted). Since an in-frame linker insertion at any given site would not necessarily inactivate the en-

TABLE 2. Linker insertion mutations in the *Nu1* and *A* genes

Name[a]	Enzyme used[b]	Linker used[c]	Site of insertion (λ bp)[d]	Gene affected
LM110	*Pvu*II	*Xho*I (12-mer)	211	*Nu1*
LM116	*Bgl*II	*Sma*I	415	*Nu1*
LM117	*Hpa*I[e]	*Sal*I	734	*A*
LM123	*Hae*III	*Xho*I (8-mer)	1107	*A*
LM124	*Hae*III	*Xho*I (8-mer)	1328	*A*
LM130	*Hae*III	*Xho*I (8-mer)	1486	*A*
LM112	*Pvu*II	*Xho*I (12-mer)	1919	*A*
LM129	*Hae*III	*Xho*I (8-mer)	1955	*A*
LM128	*Hae*III	*Xho*I (8-mer)	1999	*A*
LM127	*Hae*III	*Xho*I (8-mer)	2040	*A*
LM119	*Eco*RV	*Xho*I (12-mer)	2088	*A*
LM125	*Hae*III	*Xho*I (8-mer)	2208	*A*
LM114	*Pvu*II	*Xho*I (8-mer)	2528	*A*

[a] The pFM123 derivatives carrying these mutations are designated pLM followed by the mutation number (e.g., the plasmid carrying the LM110 mutation is called pLM110).

[b] The wild-type plasmid, pFM123, was partially or completely digested with this restriction endonuclease to construct the given linker insertion.

[c] The linker contains one or two sites for the restriction endonuclease indicated in parentheses. The sequences of the linkers are as follows: *Xho*I (12-mer), 5'-CTCGAGCTCGAG; *Xho*I (8-mer), 5'-CCTCGAGG; *Sma*I, 5'-GATCCCCGGG; *Sal*I, 5'-AAGTCGAGTT.

[d] The number indicates the base that is immediately 5' to the site of the insertion. The λ DNA sequence numbers are those of reference 35.

[e] The *Hpa*I site lies in the region of overlap between the *Nu1* and *A* genes, actually cutting in the *Nu1* stop codon; however, the linker restores the normal *Nu1* stop codon and causes a frameshift mutation only in the *A* gene.

TABLE 3. Marker rescue λ *A*am phages by *A* gene deletion plasmids

Deletion plasmid[a]	Deletion end point (λ bp)[b]	λ *A*am phage plating efficiency[c]								
		11	ps3	a1	36	19	a2	a3	854	32
pDM114	2528	+	+	+	+	+	+	+	+	+
pDM125	2209	+	+	+	+	+	+	+	−	−
pDM112	1919	+	+	+	+	±	−	−	−	−
pDM130	1487	+	±	−	−	−	−	−	−	−
pDM124	1329	±	−	−	−	−	−	−	−	−
pDM123	1108	−	−	−	−	−	−	−	−	−

[a] The number of each deletion (DM) plasmid corresponds to the linker insertion (LM) mutation plasmid from which it was made.

[b] Each plasmid carries the whole *Nu1* gene and a segment of the *A* gene starting at its 5′ end (λ bp 707) and ending at the stated deletion end point.

[c] The λ *A*am phages were plated on 594 (Sup0) cells carrying the indicated deletion plasmid. Symbols: −, the observed plating efficiency was the same as on 594 cells with no plasmid; ±, the observed plating efficiency was approximately 100-fold higher than the efficiency on 594 cells with no plasmid; +, the observed plating efficiency was at least 1,000-fold higher than on 594 cells with no plasmid.

zyme, an 8-bp linker, whose insertion causes frameshift mutations, was also used. Using this linker guaranteed that debilitating mutations at many different positions could be produced by the mutagenesis procedure.

Construction of plasmids with *A* gene deletions. Once pFM123 derivatives with *Xho*I linker insertions widely distributed across the *A* gene had been isolated, it was possible to construct a series of *A* gene deletion plasmids. The end points of these deletion plasmids, which are indicated in Table 3, correspond to sites where linkers were inserted in pFM123. It can be seen that these deletions end at regularly spaced intervals throughout the *A* gene and are thus very useful for mapping *A* gene mutations carried on phages.

Characterization of λ *A*am mutations. Nine λ *A*am phage isolates, which had been previously isolated in our and other laboratories (Table 1), were used in experiments described below to map spontaneous *A* gene mutations carried on plasmids. Before the *A*am phages could be used in these experiments, it was essential to characterize them further. The mutation carried by each phage was mapped by plating on 594 (Sup0) cells transformed by the *A* gene deletion plasmids described above. Table 3 shows the results of these experiments. It can be seen that phage mutations were rescued by the plasmids within the 594 cells and that loss of marker rescue correlated with deletions of the *A* gene. *A*am854 and *A*am32 are rescued by pDM114, but not pDM125, indicating that these phage mutations must map to the 3′ side of λ bp 2209 and to the 5′ side of bp 2528. Similar analysis places the *A*ama2 and *A*ama3 mutations between bp 1919 and 2209; the *A*ama1, *A*am36, and *A*am19 mutations between bp 1487 and 1919; the *A*amps3 mutation between bp 1329 and 1487; and the *A*am11 mutation between bp 1108 and 1329. The intermediate values (±) probably arise because the phage mutations are very close to the deletion end point.

Fragments containing the ama1, a2, a3, 19, 32, and 854 mutations were subcloned into pFM123 (Table 4, column 1) for use in the marker rescue experiments described below. These derivatives were given the name pAM followed by the number of the *A*am isolate from which they were cloned. The ama2, a3, 32, and 854 mutations were sequenced from the indicated subcloned fragments, and their exact positions are shown in Table 4. Since the approximate locations of these mutations were already known from the marker rescue experiments, it was only necessary to sequence the regions where the mutations were expected to be. The exact position of the *A*am19 mutation was deduced from its behavior in the marker rescue experiments (Table 3; see also Table 7) and from the observation that pAM19 was missing the *Pvu*II site that is normally found starting at λ bp 1917 in the *A* gene.

Isolation and initial characterization of spontaneous terminase mutations. When N205 cells carrying pFM123 are grown at a high temperature, most of the cells are killed; however, colonies which are resistant to terminase killing arise spontaneously at a frequency of about 5×10^{-5} (26). Forty-two independent cultures of N205 cells bearing pFM123 were plated at 42°C, and one to four surviving colonies were picked from each plate. In total, 94 surviving colonies were picked. To determine whether cell survival was due to a plasmid mutation, we isolated plasmid DNA from cultures of each surviving colony and used it to transform N205 cells. Cells transformed by mutant plasmids would not be killed when plated at 42°C. When this analysis was performed, it was found that 77 of the 94 plasmids isolated were unable to kill N205 cells. The 17 isolates that contained wild-type plasmids presumably survived because of a mutation in the host. Cells that are *recA* and also IHF$^-$ (*himA* or *himD* [*hip*] mutants) can survive terminase treatment (26). Of the 17 mutants isolated here, however, 10 were still able to support the growth of phage 21 (which requires integration host factor [IHF]) and were presumed to be IHF$^+$. These putative *E. coli* mutants have not been further characterized.

TABLE 4. Sequencing and subcloning of *A*am mutations

Name	Fragment subcloned into pFM123[a]	Fragment subcloned for sequencing[b]	Mutation[c]	Amino acid changed[d]
*A*ama1	*Bgl*II-*Bss*HII (415–3522)	ND[e]	ND	ND
*A*am19	*Bgl*II-*Bss*HII (415–3522)	ND	C-1917 → T[f]	Gln-403
*A*ama2	*Hpa*I-*Sph*I (734–2212)	*Bgl*II-*Sph*I (415–2212)	C-1986 → T	Gln-426
*A*ama3	*Hpa*I-*Sph*I (734–2212)	*Bgl*II-*Sph*I (415–2212)	G-1972 → A	Trp-421
*A*am854	*Sph*I-*Bss*HII (2212–3522)	*Sph*I-*Sph*I (2212–12002)	C-2403 → T	Gln-565
*A*am32	*Sph*I-*Bss*HII (2212–3522)	*Sph*I-*Sph*I (2212–12002)	G-2416 → A	Trp-569

[a] In each case the indicated fragment which contained the amber mutation was used to replace the corresponding fragment in pFM123. The numbers in parentheses refer to the position of the site in the λ DNA sequence (35).

[b] The indicated restriction enzyme fragments were cloned from mature λ DNA isolated from each mutant phage into pTZ18R cut with either *Bam*HI and *Sph*I or just *Sph*I.

[c] The number in parentheses indicates the position of the mutated base in the λ DNA sequence.

[d] The number shown indicates the position of the residue within the gpA amino acid sequence as deduced from the DNA sequence.

[e] ND, not done.

[f] The position of this mutation was deduced from genetic and restriction mapping data (see text).

The 77 mutant plasmids were assigned numbers and given the name pTM. Analysis of these plasmids showed that some of those isolated from the same culture were likely to be siblings. These duplications were eliminated. Ultimately, the total number of different plasmid mutations analyzed was 63. To detect any gross rearrangements or deletions, the mutant plasmids were digested with various combinations of the restriction enzymes shown in Fig. 1. DNA fragment sizes were determined by electrophoresis on 2% agarose gels, so that most insertions or deletions 50 bp or greater would have been detected. Some rearrangements that did not change fragment sizes might have been missed. Among the 63 plasmid mutations examined, 17 had visibly altered restriction digest patterns (data not shown). In all cases but one, these alterations were the result of large (more than 500 bp) insertions or deletions in the terminase genes or in the promoter region. One deletion of less than 50 bp was detected in the *A* gene because it removed a restriction enzyme site.

In vitro packaging activities of terminase mutants. To evaluate the effects of the terminase mutations and to help determine which terminase gene was mutated, extracts made from cells carrying mutant plasmids were assayed for their ability to package λ DNA in vitro. The levels of gpNu1 and gpA activity were measured separately. N205 cells transformed by pLM116 and pLM117, which have linker insertion-induced frameshift mutations in their *Nu1* and *A* genes, respectively, were grown and tested in every assay as controls. AZ1544 (a $recA^+$ derivative of N205) cells carrying pFM123 ($Nu1^+$ A^+) were also grown as a control (AZ1544 had to be used in this case because N205 transformed with pFM123 would not grow at 42°C). Table 5, group A, shows that the plasmid carrying both wild-type genes gave the highest values in both the gpNu1 and gpA assays. When either subunit was mutated, a drop in the activity of the other subunit was observed. This phenomenon was especially striking in the case of the *Nu1* mutant, pLM116, which produced almost 30-fold less gpA activity than pFM123 did. The lowered gpA activity of the pLM116 extract probably was due to the instability of gpA in the absence of gpNu1. In vitro experiments in our laboratory have shown that wild-type gpA alone is very labile at 42°C, but is stabilized upon mixing with gpNu1 (unpublished results). Lowered activity of one subunit in extracts of cells transformed by plasmids in which the other subunit gene is mutated might also be expected if the gpNu1-gpA complex does not form as efficiently in vitro as it does in vivo. In assessing whether a particular plasmid was mutated in its *Nu1* or *A* gene, it was important to compare the packaging activity of its extract with that of the pLM117 or pLM116 extracts as well as the wild-type (pFM123) extract. The gpNu1 and gpA activities of extracts made from each of the spontaneous mutant plasmids without visible DNA rearrangements and the in-frame linker insertion mutants were measured, and the results are shown in Tables 5 and 6.

Table 5, group B, shows the results for extracts which had greater decreases in gpNu1 activity than in gpA activity. The reductions in gpNu1 activity ranged from 10- to 10^5-fold. Although the gpA activities observed in this group were significantly lower than in the pFM123 extract, they were in most cases higher than in the pLM116 extract. The gpA produced by these plasmids may be more stable than that produced by pLM116 because they are also still making a relatively large amount of gpNu1. It can be seen that the level of gpA activity of the extracts in this group decreased as the level of gpNu1 activity decreased until it approximated the level of pLM116. The plasmids tested in group B probably contain *Nu1* mutations, or mutations that affect the expression of the *Nu1* gene; however, no further genetic or DNA sequencing studies were performed to confirm this hypothesis.

TABLE 5. In vitro packaging activities of mutant plasmid extracts

Group	Extract[a]	Relative packaging activity[b]	
		gpNu1	gpA
A	pFM123 ($Nu1^+A^+$)	1.0	1.0
	<u>pLM116</u> ($Nu1^-A^+$)	$<10^{-5}$	3.5×10^{-2}
	<u>pLM117</u> ($Nu1^+A^-$)	4.0×10^{-1}	$<10^{-6}$
	No terminase	$<10^{-5}$	$<10^{-6}$
B	pTM42	8.5×10^{-2}	1.2×10^{-1}
	pTM161	5.1×10^{-2}	1.0×10^{-1}
	pTM381	3.3×10^{-2}	2.4×10^{-1}
	pTM232	3.2×10^{-2}	1.8×10^{-1}
	pTM511	2.0×10^{-2}	3.3×10^{-1}
	pTM71	1.8×10^{-2}	3.6×10^{-1}
	pTM212	1.6×10^{-2}	2.3×10^{-1}
	pTM342	1.4×10^{-2}	2.2×10^{-1}
	pTM532	1.1×10^{-2}	9.0×10^{-2}
	pTM11	7.4×10^{-3}	9.6×10^{-2}
	pTM62	6.8×10^{-3}	7.4×10^{-2}
	pTM151	4.2×10^{-4}	1.1×10^{-2}
	pTM201	$<10^{-5}$	5.2×10^{-2}
	<u>pLM110</u>	$<10^{-5}$	1.0×10^{-2}
C	pTM341	2.8×10^{-2}	9.0×10^{-4}
	pTM541	2.2×10^{-3}	5.0×10^{-5}
	pTM233	1.2×10^{-3}	2.8×10^{-4}
	pTM101	$<10^{-5}$	5.5×10^{-6}
	pTM262	$<10^{-5}$	2.7×10^{-6}
D	pTM311	2.8×10^{-4}	5.7×10^{-3}
	pTM12	1.3×10^{-4}	7.7×10^{-3}
	pTM213	8.5×10^{-5}	8.7×10^{-4}

[a] An extract made from a particular plasmid-transformed cell is referred to only by the name of the plasmid; for example, an extract made from N205 cells transformed by pLM110 is referred to as a pLM110 extract. Most of the plasmids tested contained spontaneous mutations. The linker insertion plasmids tested are underlined.

[b] Packaging activity was measured by using mature λ DNA as the substrate; the results are expressed relative to the levels obtained with the wild-type extract (pFM123), which are assigned the value of 1.0.

The pTM extracts assayed in group C were significantly reduced to various degrees in both their gpA and gpNu1 activities; thus, locations of the mutations in these plasmids are uncertain. It is conceivable that a mutation in one terminase subunit could lead to a reduction in the activity of the other subunit if the mutant subunit were able to make a very stable complex with the nonmutated subunit. However, marker rescue experiments, as described below, did not detect *A* gene mutations in any of these plasmids (data not shown). The most likely explanation for the phenotypes of these plasmids is that they have mutations in their promoter regions or elsewhere on the plasmid that affect the expression of both terminase genes. The extracts tested in group D were made from plasmids with insertions in their promoter regions, and, like the group C plasmids, they also show reductions in the activities of both subunits.

Table 6 contains the results obtained with extracts of plasmids that were shown, in packaging assays and in the genetic tests described below, to have *A* gene mutations. When mature λ DNA was the substrate, most of these

TABLE 6. In vitro packaging activities of *A* mutants

Extract	Relative gpNu1 activity[a]	gpA protein production[b]	Relative gpA activity		
			Mature DNA[a]	Immature DNA[c]	High temperature[d]
pFM123	1.0	+	1.0	1.0	1.0
pTM6	8.8×10^{-1}	+	1.5×10^{-1}	1.5×10^{-5}	3.6×10^{-2}
pTM33	5.6×10^{-1}	+	1.1×10^{-1}	8.0×10^{-5}	1.6×10^{-3}
pLM112	4.8×10^{-1}	ND	7.4×10^{-2}	4.0×10^{-6}	2.4×10^{-5}
pTM211	4.4×10^{-1}	+	5.8×10^{-2}	4.6×10^{-3}	3.0×10^{-4}
pTM122	8.8×10^{-1}	–	5.3×10^{-2}	1.8×10^{-3}	2.0×10^{-5}
pTM503	3.7×10^{-1}	+	1.9×10^{-2}	1.7×10^{-3}	1.6×10^{-3}
pLM114	3.5×10^{-1}	ND	2.7×10^{-3}	3.7×10^{-4}	3.5×10^{-5}
pTM471	3.0×10^{-1}	–	1.4×10^{-4}	–[e]	–
pTM181	3.6×10^{-1}	+	4.8×10^{-5}	–	–
pTM152	7.2×10^{-1}	–	3.2×10^{-5}	–	–
pTM331	2.1×10^{-1}	+	1.7×10^{-5}	–	–
pTM222	3.8×10^{-1}	+	3.9×10^{-6}	–	–
pLM119	2.8×10^{-1}	+	$<10^{-6}$	–	–
pTM21	3.7×10^{-1}	+	$<10^{-6}$	–	–
pTM81	3.9×10^{-1}	–	$<10^{-6}$	–	–
pTM91	3.4×10^{-1}	+	$<10^{-6}$	–	–
pTM121	6.0×10^{-1}	–	$<10^{-6}$	–	–
pTM131	4.0×10^{-1}	–	$<10^{-6}$	–	–
pTM142	5.2×10^{-1}	–	$<10^{-6}$	–	–
pTM162	4.0×10^{-1}	+	$<10^{-6}$	–	–
pTM202	4.4×10^{-1}	–	$<10^{-6}$	–	–
pTM204	4.8×10^{-1}	+	$<10^{-6}$	–	–
pTM221	3.6×10^{-1}	–	$<10^{-6}$	–	–
pTM231	3.0×10^{-1}	–	$<10^{-6}$	–	–
pTM252	2.4×10^{-1}	+	$<10^{-6}$	–	–
pTM281	2.8×10^{-1}	–	$<10^{-6}$	–	–
pTM332	2.2×10^{-1}	–	$<10^{-6}$	–	–
pTM401	2.4×10^{-1}	–	$<10^{-6}$	–	–
pTM441	2.2×10^{-1}	+	$<10^{-6}$	–	–
pTM442	5.6×10^{-2}	+	$<10^{-6}$	–	–
pTM501	1.9×10^{-1}	–	$<10^{-6}$	–	–

[a] Activities were measured with mature λ DNA as the substrate; the results are expressed as in Table 5.
[b] Results are taken from the data in Fig. 2 and are shown here for the sake of comparison.
[c] Activities were measured with immature λ DNA as the substrate; the results are expressed relative to the activity of the pFM123 extract on this substrate. The activity of the pFM123 extract with this preparation of immature λ DNA was approximately 10-fold lower than it was with mature λ DNA.
[d] Activities were measured with mature λ DNA as the substrate, but the reactions were carried out at 37°C instead of room temperature. The results are expressed relative to the activity of the pFM123 extract at this temperature. The pFM123 extract activity was reduced about 10-fold at the high temperature compared with the activity at room temperature.
[e] –, assays with immature DNA and at high temperature were not performed with these extracts.

extracts showed relatively small reductions in gpNu1 activity and extremely large reductions in gpA activity. In fact, the majority had no detectable in vitro gpA activity (denoted by $<10^{-6}$ in the table). On the other hand, a few extracts were still able to package mature DNA quite efficiently; thus, they were tested under different conditions.

Table 6, column 5, shows the gpA activity of some of the mutant extracts with immature λ DNA as the packaging substrate. Since the complete packaging of immature λ DNA requires the terminase-mediated endonuclease and packaging activities, whereas only the packaging activity is required when mature DNA is used, we reasoned that mutants lacking only endonuclease activity might still package mature DNA efficiently but be unable to package immature DNA. It can be seen that the pTM6, pTM33, and pLM112 extracts did package immature DNA 10^3- to 10^4-fold less efficiently than they packaged mature DNA and therefore that they may be specifically debilitated in their endonuclease activity. In contrast, the other mutant extracts tested showed only 10- to 30-fold differences in their abilities to package mature versus immature DNA. Assays for gpNu1 activity with immature DNA as the substrate were also performed with the same seven extracts, but none deviated significantly from the controls (data not shown).

As is seen in Table 6, column 6, gpA activity was also measured at high temperature. The rationale for performing these tests was that the mutations were selected at 42°C; therefore, some of them might display more dramatic effects when assayed at a higher temperature. This was shown to be true for the pLM112 and pTM122 extracts, which packaged 10^3-fold less efficiently at 37°C than at room temperature. The pTM211, pLM114, and pTM33 extracts were 70- to

190-fold less active when assayed at the high temperature, whereas the pTM6 and pTM503 extracts were affected only slightly by the high temperature. None of these extracts displayed altered gpNu1 activities when assayed at the high temperature (data not shown).

The pTM mutant extracts listed in Table 5, group B, which were not reduced more than 100-fold in gpNu1 or gpA activity were also tested for their ability to package immature DNA at room temperature and mature DNA at 37°C. Both gpNu1 and gpA assays were performed under these conditions, but no significant deviations from the controls were seen (data not shown).

Protein production from mutant *A* genes. Since the *A* gene mutations that we isolated were most numerous and displayed the most striking phenotypes in in vitro packaging experiments, we decided to study these in more detail. To gain some insight into the cause of the large decreases in gpA activity seen with many of the isolated mutants, we examined the levels of gpA protein produced from these mutant genes.

Under their normal translational control, the terminase subunits are among the most poorly expressed genes in *E. coli*; this makes it impossible to estimate gpNu1 and gpA production from pFM123 and its mutant derivatives by analyzing crude extracts on SDS-PAGE gels stained by normal procedures. Since it has, as yet, not been possible to obtain suitable terminase antisera, Western immunoblot analysis could not be undertaken either. For this reason, all the spontaneous *A* gene mutants shown in Table 6 and one of the in-frame linker insertions (LM119) were subcloned into a gpA-overproducing plasmid called pCM2 (27). In this way, it could be determined whether each mutant gene was capable of directing synthesis of full-length gpA at close to wild-type levels when a strong translation initiation site was provided.

Figure 2A shows SDS-PAGE gels of lysates made from cells carrying overproducing constructs containing TM mutations, which caused the complete loss of in vitro packaging activity. A lysate of cells carrying pLM117, which has a frameshift mutation in the *A* gene as a result of its linker insertion, was used as a negative control. It can be seen that normal or near-normal levels of gpA were produced from 7 of these 18 mutant *A* genes. Similarly, Fig. 2B shows that 7 of 10 plasmids carrying TM mutations which led to partial debilitation of in vitro packaging activity produced normal amounts of gpA, as did the plasmid carrying the LM119 mutation. The results of these experiments are summarized in Table 6, column 2.

Mapping *A* gene mutations on plasmids by marker rescue with *A*am phages. Since the *A* gene is relatively large (more than 1,900 bp), complete sequencing of each and every isolated mutant gene could be very laborious; therefore, an efficient method of genetically mapping mutations within the *A* gene was developed as a preliminary strategy. It was previously observed in our laboratory that when *A*am phages were plated on Sup0 cells transformed by pFM123 (which carries the wild-type *A* gene), plaques were produced at an efficiency of approximately 10^{-3} compared with the plating efficiency on amber suppressor strains. Since no terminase is expressed from this plasmid at 30°C, the temperature at which the experiment was conducted, this rescue presumably arose from recombination between the plasmid and phage, which led to the replacement of mutant phage DNA with wild-type plasmid DNA. It was surmised that a plasmid with a mutation in the *A* gene very close to the site of a particular *A*am phage mutation might rescue this mutation much less efficiently and that the magnitude of this decrease could be used to map plasmid mutations.

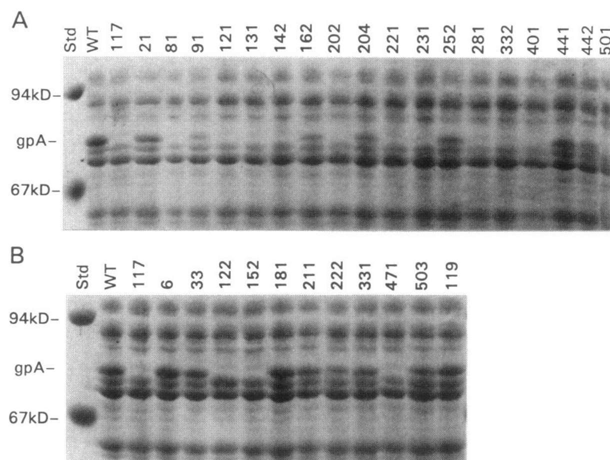

FIG. 2. Protein production directed by gpA-overproducing plasmids containing *A* gene mutations. Cells transformed by gpA-overproducing plasmids containing *A* gene mutations were grown, lysed, and separated on SDS-PAGE gels as described in Materials and Methods. Only the sections of the gels in the size range of full-length gpA are shown. The numbers above each lane refer to the isolation number of the mutation carried in the plasmid construct being tested. All the mutations tested were spontaneous (TM) mutations, except the lane marked 117, which refers to pLM117, and the lane marked 119, which refers to an overproducing construct carrying the LM119 mutation. The lanes marked WT show lysates from cells containing pCM2, the plasmid which overproduces wild-type gpA. (A) Mutations that led to total loss of in vitro gpA activity; (B) mutations that caused only partial loss of gpA activity (except lane 119). The gels shown were stained with Coomassie blue by standard procedures.

Table 7 shows the results of plating the nine different λ *A*am phages on cells carrying plasmids with spontaneous linker insertion and amber mutations in the *A* gene carried on pFM123. It can be seen that rescue efficiency was lower than that in the wild type on all the mutant plasmids tested; however, plasmids, such as pLM116 or pLM117, with mutations far from any of the phage mutations showed smaller reductions and rescued each phage with almost the same efficiency. Most of the mutant plasmids displayed a marked reduction in rescue efficiency with at least one phage because the plasmid mutation was close to this phage mutation. As would be expected, the lowest marker rescue efficiencies were obtained when a particular λ *A*am phage was plated on a plasmid carrying exactly the same mutation. The values observed in these cases were the same as when the phage was plated on a Sup0 strain lacking any plasmid. It can be seen that marker rescue efficiency generally increased as the physical distance between the plasmid and phage mutations involved increased.

The rescue frequencies observed between plasmid and phage mutations in known positions could, in general, be correlated with the physical distance separating them. For example, rescue efficiencies of greater than 10% were seen only when the phage and plasmid mutations were more than 100 bp apart, and rescue frequencies of 5% or less were observed only when the plasmid and phage mutations were less than 100 bp apart. Significant levels (1 to 2%) of rescue were observed even when the plasmid and phage mutations were less than 15 bp apart. The effect of a plasmid mutation

TABLE 7. Marker rescue of λ *A*am phages by cells carrying plasmids with *A* gene mutations

Mutation	Known mapping positions (λ bp)[b]	Relative plating efficiency of λ *A*am phages[a]									Estimated positions (λ bp)[d]
		11 (1320)[c]	ps3 (1450)	a1 (1650)	36 (1850)	19 (1917)	a3 (1972)	a2 (1986)	854 (2403)	32 (2416)	
FM123	Wild type	100	100	100	100	100	100	100	100	100	
LM116	415	29	41	45	49	49	44	44	44	40	
LM117	734	27	27	32	35	41	34	28	33	38	
TM281		30	25	34	30	35	34	32	38	32	
TM501		28	26	31	30	36	36	38	28	29	
TM81		26	24	32	32	23	24	27	35	37	700–1100
LM123	1108	23	23	38	31	43	29	33	38	35	
TM222		16	20	42	27	27	27	38	40	33	
TM331		10	16	18	15	15	17	15	24	28	1000–1200
TM162		8	12	16	19	15	20	17	20	20	
TM401		6	13	16	16	19	19	18	21	24	
TM442		6	11	14	15	21	17	18	18	23	1200–1350
TM21		3	10	15	14	18	17	18	20	23	
LM124	1329	0.3	13	15	20	20	21	18	24	29	
LM130	1487	15	3	12	16	17	17	18	20	26	
TM91		15	2	14	12	21	17	17	21	23	
TM204		16	2	13	18	21	17	21	25	19	
TM231		16	6	13	13	18	17	17	20	28	1400–1550
TM252		16	4	15	17	21	21	23	28	25	
TM503		13	6	17	18	20	17	20	25	27	
AMal		23	12	0.1	8	10	17	12	18	27	1550–1750
TM152		15	13	0.1	7	14	10	13	20	16	
TM131		12	12	5	5	11	12	12	20	17	1700–1800
TM221		19	13	11	5	10	13	12	18	21	1750–1850
TM332		21	11	11	2	12	12	12	21	24	
TM202		15	16	11	0.3	5	6	10	17	17	1825–1875
AM19	1917	20	9	11	4	<0.1	4	5	12	19	
LM112	1919	19	10	10	4	<0.1	3	3	12	13	
TM6		23	11	10	4	2	4	2	14	19	1900–1950
TM181		17	14	10	4	1	3	2	11	15	
LM129	1956	16	11	11	8	3	4	6	17	16	
AMa3	1972	19	13	12	6	4	0.2	1	13	15	
TM471		19	13	14	7	8	<0.1	2	13	14	1970–1975
AMa2	1986	19	10	12	6	3	2	0.2	10	13	
LM128	2000	24	13	13	12	7	4	2	17	18	
LM127	2041	20	14	13	13	8	5	9	19	19	
TM142		26	16	18	9	11	5	5	18	19	2000–2100
LM119	2088	29	13	14	13	11	8	5	10	11	
TM121		27	17	20	13	11	7	5	10	11	2100–2300
TM441		25	15	18	16	14	10	7	10	10	
LM125	2209	24	18	18	17	12	10	8	10	14	
AM854	2403	30	17	21	16	18	16	10	0.1	0.7	
AM32	2416	34	16	20	15	14	17	10	1	0.1	
TM122		33	19	21	18	17	14	12	4	3	2350–2475
TM33		34	19	18	17	18	22	12	3	4	
LM114	2528	24	20	24	18	22	14	14	8	6	
TM211 (42°C)		34	22	34	25	18	16	19	7	9	2500–2635

[a] Plating efficiencies were derived by dividing the number of plaques observed on a particular plate by the number of plaques observed when the same phage was plated on cells carrying the wild-type plasmid (pFM123) and then multiplying by 100.

[b] The values for plasmids with mutations of known position (linker insertions or sequenced amber mutations) are boxed.

[c] The numbers in parentheses following the phage mutation numbers are their mapping positions in the λ genome in λ base pairs. The positions of *A*am11, a19, a1, and 36 are approximations based on previous genetic mapping and the results in this table. The positions of the other mutations are known exactly from sequencing or restriction mapping.

[d] These values were derived by comparing the results obtained by plating on cells carrying plasmids with mutations in unknown positions with those obtained with the mutations whose positions were known (see text).

on rescue efficiency could still be detected even when the closest phage mutation was almost 200 bp away (e.g., the LM125 mutation); however, the rescue efficiency when mutations were 100 bp apart was not significantly different from that when mutations were 200 bp apart. Therefore, unknown plasmid mutations far from an accurately mapped phage mutation (e.g., TM152 and pAMal) could not be mapped with precision.

All the mutations listed in Table 7 were assigned approximate mapping positions by comparing their patterns of rescue efficiency with those of the plasmids carrying mutations in known positions and by using the general observations mentioned above. In assigning mapping positions from the marker rescue data, it was assumed that the recombination rate across the *A* gene is approximately uniform, as it has been shown that the λ *red* system (which was presum-

ably mediating recombination in this case since the cells were *recA*) promotes exchanges with nearly uniform frequency across the λ genome as long as the DNA is being replicated (for a review, see reference 37). It was possible to map some mutations more accurately than others because they were very close to phage mutations. For example, the TM471 mutation probably maps in the same codon as the ama3 mutation since it could not rescue λ *A*ama3 at all. Mutations lying within the last 600 bp of the *A* gene could generally be mapped with the most confidence because of the larger number of accurately mapped phage and plasmid mutations in this region, whereas mutations in the first 600 bp of the gene were difficult to localize because none of the phage *A*am mutations mapped in this region. The sizes of the intervals in the mapping positions were estimated by examining the efficiency of rescue between the plasmid mutation and the closest phage mutations and comparing these values with those found in the cases in which the distance between the phage and plasmid mutations was known exactly (i.e., the cases in which both the plasmid and phage mutations had been sequenced or mapped by restriction analysis).

The marker rescue experiments were performed at 30°C to avoid complications that might have resulted from terminase expression from the plasmid; however, it was possible that some mutations manifested themselves only at high temperature. Therefore, mutations which could not be accurately mapped when the marker rescue experiment was performed at 30°C were also tested at 42°C. There was no significant difference in the results for any mutation except TM211, which did not seem to map closely to any phage mutation when tested at 30°C, but did at 42°C. The pTM211 results shown in Table 7 are those from the 42°C experiment.

The marker rescue data made it possible to map some of the unsequenced *A*am mutations more exactly. The plating efficiency of λ *A*am11 on pLM124 was only 0.3%, which is similar to the efficiencies observed when other λ *A*am phages were plated on plasmids carrying the same amber mutation; thus, it can be concluded that the am11 mutation is probably no more than 10 bp away from λ bp 1329. Similar reasoning led to the conclusion that the *A*amps3 mutation is not likely to be more than 50 bp from λ bp 1487, since it was rescued with only 3% efficiency on pLM130. The plating of λ *A*am19 on pLM112 (an insertion at bp 1919) produced no plaques, which is in agreement with the assignment of the am19 mutation to bp 1917. λ *A*ama1 is the only amber mutation that was difficult to map, because it seems to be more than 100 bp from any of the known plasmid mutations.

DISCUSSION

The main objective of this work was to isolate and characterize λ terminase gene mutations that would aid in the elucidation of the location and function of the many activity domains encoded in this complex protein. To achieve this goal, we analyzed 46 plasmids with different spontaneous mutations which were not the result of detectable DNA rearrangements such as deletions or insertions. Linker insertion mutations at 13 different positions in the *Nu1* and *A* genes were also isolated. This work focused on the characterization of these 59 mutant plasmids.

In vitro characterization of mutant extracts. In vitro λ DNA-packaging assays were performed with mutant extracts to gauge the degree of activity reduction caused by the mutations and to determine which terminase subunit was affected. Although we assume that the mutants were selected for their inability to cut DNA, packaging is a much more sensitive and reliable assay for gpNu1 and gpA activity in crude extracts. In addition, we anticipated that most mutations causing a reduction in cutting would also reduce packaging to some extent, since most of the terminase activities required for cutting, such as DNA binding and subunit interaction, are also required for packaging.

From the results of the in vitro packaging assays, it was possible to determine that 28 of the spontaneous mutant plasmids contained a mutation in gene *A* (Table 6) and that 13 contained a mutation in *Nu1* (Table 5, group B). The number of *Nu1* mutants is somewhat tentative because some of them did not show large decreases in packaging activity and because the mapping positions of the mutations have not yet been confirmed by genetic studies or DNA sequencing. The *A* mutations generally have more severe effects on packaging activity than the putative *Nu1* mutations do. Whereas only 4 of the 13 putative *Nu1* mutations caused more than a 100-fold reduction in gpNu1 activity, the majority of the putative *A* gene mutations led to at least 10^4-fold reductions in activity. At this time, it is uncertain whether this observation is due to chance or to some intrinsic property of the *Nu1* gene or protein.

It is evident from the in vitro packaging results that the level of terminase being produced from a mutant plasmid does not have to be too drastically reduced for the cell to survive. This phenomenon can be seen most clearly for pTM42 in Table 5, group B, for which only a 10-fold drop in gpNu1 and gpA activity is observed. This mutant extract, and others which did not show large reductions when packaging mature DNA at room temperature, also displayed a similar pattern of activity when tested at high temperature and with immature DNA (data not shown). This finding is consistent with the previous observation that *recA* cells which are mutated in one or both of the genes encoding IHF are resistant to killing by terminase (26). IHF is required for optimal terminase activity in vitro and in vivo (12, 16); however, wild-type λ can still grow in IHF$^-$ cells and in vitro packaging is decreased by only 10- to 30-fold when IHF$^-$ extracts are used (12; our unpublished observations). It appears that reducing the terminase activity by approximately 10-fold, whether by mutation in one of the terminase genes on the plasmid or by a mutation in an IHF gene, is sufficient to spare *recA* cells from the lethal effect. It should be noted that the cutting of a *cos* site on wild-type phage λ is not necessarily equivalent to the cutting of a cryptic *cos* in the *E. coli* genome. For example, a recent study has shown that the *cos* site of the *QSR'* cryptic prophage has different properties from a wild-type *cos* site in that phages bearing the *QSR'-cos* are able to grow on IHF$^-$ HU$^-$ cells whereas wild-type λ phage is not (25).

Mutations in the putative endonucleolytic domain of terminase. Extracts of pTM6, pTM33, and pLM112 showed 10^3- to 10^4-fold decreases in in vitro gpA activity when immature λ DNA was the packaging substrate (Table 6), whereas their activities were reduced by only approximately 10-fold when mature λ DNA was packaged. Thus, these mutant terminases appear to be specifically damaged in their ability to package uncut λ DNA. Their mutations are likely to lie within the domain or domains of terminase responsible for its endonucleolytic activity. These mutations lie in two distinct locations. The TM6 mutation was mapped to a position in the *A* gene near the LM112 insertion mutation which lies at λ bp 1919 (Table 4). pTM33, on the other hand, mapped about 400 bp further downstream, closer to the 3′ end of the gene. It is interesting that these mutations lie on either side of a putative ATP-binding consensus sequence in

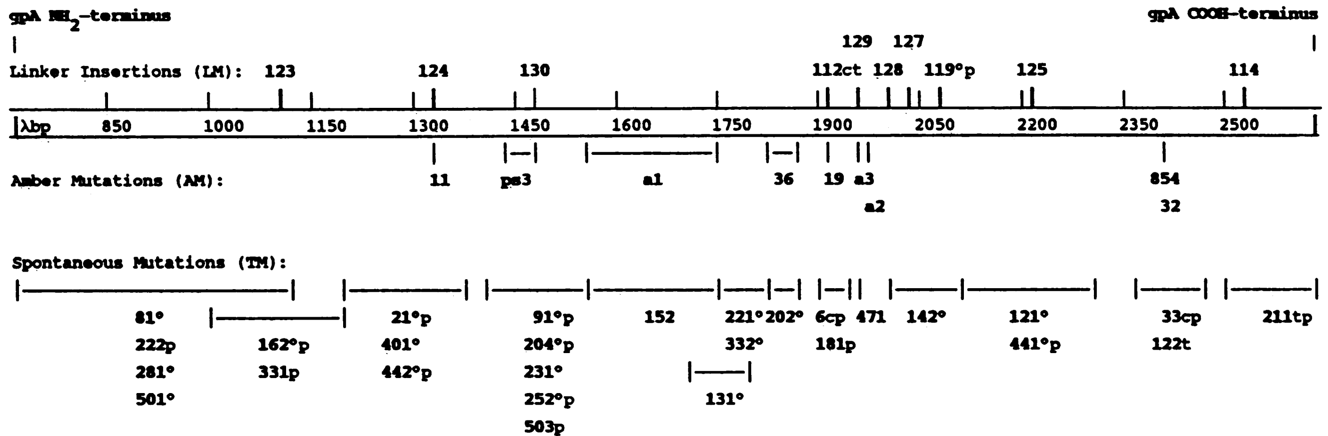

FIG. 3. Location of *A*am, linker insertion, and spontaneous mutations in the *A* gene. This map represents λ DNA from bp 700 to 2700 (numbering is that of reference 35). The lines above the number of the spontaneous mutations represent their estimated mapping positions. The mutation numbers marked with superscript o displayed no gpA activity in in vitro packaging; those marked with c packaged only mature DNA efficiently; those marked with t displayed extreme temperature sensitivity in vitro, and those marked with p produced detectable amounts of full-length protein on SDS-PAGE gels.

the *A* gene (17), since it has been shown that an interaction with ATP is essential for *cos* cleavage in vitro (15, 18). These mutations provide the first clue to the localization of the terminase domain(s) responsible for its endonucleolytic activity. Interestingly, a mutation in the gene encoding the large subunit of the phage T3 packaging enzyme also causes the loss of endonuclease activity without affecting the packaging activity. Similar to the mutants described here, this mutant protein could mediate efficient packaging of mature DNA, but was unable to package immature DNA (20).

Protein production from mutant *A* genes. Because the protein production from mutant *A* genes could not be examined directly, it was necessary to subclone the genes into a gpA-overproducing plasmid. The results of this analysis must be viewed with some caution since the mutant *A* genes are being expressed in a different context from the one in which they were isolated and assayed. Many of the mutations in the *A* gene led to the loss of detectable protein production from the mutant genes. This could be due to frameshift mutations, nonsense mutations, small deletions that were undetectable in the restriction enzyme analyses, or mutations that lower the efficiency of transcription and/or translation. Some of these mutants may contain point mutations that lead to the production of structurally unstable proteins. It has been shown that such proteins are often rapidly degraded in *E. coli* (33). Three mutations, TM122, TM152, and TM471, caused the loss of detectable protein production, yet extracts of cells carrying plasmids with these mutations still had detectable levels of gpA activity in the in vitro packaging assays. It is likely that these mutant proteins are unstable and are being degraded in the cell, but there is still enough protein left in the extracts to be detected in the packaging assay. The temperature sensitivity of the gpA activity of the pTM122 extract supports this hypothesis.

Of the 29 *A* gene mutations tested, 15, including the LM119 linker insertion mutation, produced normal or near-normal levels of full-length protein (Fig. 2). No attempt was made to quantitate the exact amount of protein produced by each of these mutants, but it is unlikely that any of these mutations caused more than a two- to threefold decrease in protein production, since reductions of this magnitude would have been detected on the gels that were run. The mutant genes which still produce protein are likely to be point mutations. Since these mutant proteins are not degraded to any appreciable extent, they are probably stably folded. Thus, they may contain mutations that affect only one gpA activity domain.

Genetic mapping of *A* gene mutations. To determine the distribution of the numerous *A* mutations, we used an efficient mapping technique based on marker rescue. The results of this analysis, summarized in Fig. 3, showed that the spontaneous mutations are fairly evenly distributed across the gene, except for some clustering near λ *A*amps3. It is possible that some of the mutations in this region, for example TM91, TM204, and TM252, are all the same mutation in a mutational hot spot, since they have the same in vitro phenotypes and behave similarly in the marker rescue experiments. Mutations associated with production of full-length protein map in many locations, which indicates that point mutations affecting various gpA domains have been isolated.

This work has shown that informative mutations within the λ terminase genes can be isolated, characterized, and mapped by simple techniques. Using these techniques, we have established the first large collection of mutations within genes encoding a bacteriophage packaging enzyme. In addition, we have found mutations which seem to specifically abolish the endonuclease activity of terminase. At this time, it is not known what terminase domains might be affected by the other mutations isolated in this work. Since these mutations were presumably selected for their inability to cut cryptic *cos* sites in the *E. coli* chromosome, they could be found in any of the terminase domains which are required for *cos* cleavage. These domains include those responsible for DNA binding, subunit assembly, ATP binding, and possibly other as yet unidentified activities. Since this selection system allowed us to isolate a group of mutations with a wide spectrum of phenotypes, it seems likely that many will prove to be useful in further functional and structural studies of terminase.

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