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An Accessory Role for *Escherichia coli* Integration Host Factor: Characterization of a Lambda Mutant Dependent Upon Integration Host Factor for DNA Packaging

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Bacteriophage lambda grows lytically on *Escherichia coli* defective for integration host factor, a protein involved in λ site-specific recombination and the regulation of gene expression. We report the characterization of a mutant, λ *cos*154, that, unlike wild-type λ, is defective for growth in integration host factor-defective *E. coli*. The *cis*-dominant mutation in λ *cos*154 is a single base pair change in a region of hyphenated dyad symmetry close to the λ left cohesive end; this mutation prevents DNA packaging. We propose the following two alternative roles for this site in λ DNA packaging: (i) to bind an *E. coli* accessory protein required in the absence of integration host factor or (ii) to bind the phage-encoded terminase protein that is essential for DNA packaging.

The bacteriophage lambda is encapsidated as a linear 48.5-kilobase duplex DNA molecule by cleavage of a long concatemer formed late in infection by rolling circle replication (Fig. 1) (reviewed in references 6 and 14). This cleavage occurs at a unique site called *cos* and requires the phage terminase protein encoded by the genes *Nu*1 and *A* (16). The terminase protein introduces two nicks in the DNA separated by 12 base pairs (bp) and generates 5′ single-strand extensions known as the λ cohesive ends (2, 42). On infection of *Escherichia coli* by λ, the linear DNA is injected, the cohesive ends pair, and the circularized DNA is ligated to form a covalently closed molecule to serve as a substrate for replication (39).

The scanning model of DNA packaging (8) suggests that, after the initial cleavage at *cos*, the terminase protein remains bound to the prohead as the λ DNA is encapsidated. When terminase encounters a second *cos* on the DNA moving into the prohead, it cleaves the DNA again (Fig. 1). Recent studies have defined the substrate surrounding *cos* that is required for in vivo cleavage and packaging. A 160-bp region extending from about bp −40 from the left of the *cos* junction to bp +120 to the right appears both necessary and sufficient for efficient encapsidation (9, 19, 33).

Both the *cos* cleavage reaction and the encapsidation of phage DNA can be accomplished in vitro (1, 21, 40). These reactions require phage DNA, terminase protein, ATP, and extracts of *E. coli* (2). This suggests the participation of host protein(s) in the reaction. A 22-kilodalton (kd) protein purified from uninfected cellular extracts promotes *cos* cleavage in vitro (16). Recent findings indicate that purified integration host factor (IHF) also supports this in vitro reaction (16).

IHF binds to DNA and is isolated in active form as a 1:1 multimer of the products of the *E. coli himA* and *hip* (*himD*) genes (32, 36; E. Flamm, personal communication). IHF was identified as an accessory factor in the site-specific recombination reactions for integration and excision of the λ genome (23). These recombination reactions, which occur between unique sites on the λ and *E. coli* DNA, require the phage-encoded Int protein (3, 35). Although IHF has no DNA cleavage activity, it binds to specific sequences on the λ DNA near the sites of recombination and enhances the binding of the Int protein (36). In another capacity, IHF controls the expression of a variety of genes (11, 22, 26, 30).

Does IHF participate in the λ DNA packaging reaction? Although it has been shown that IHF promotes *cos* cleavage in vitro (14), in vivo studies demonstrate that λ can grow lytically on either *himA* or *hip* mutants (27). This indicates that, in vivo, λ does not utilize IHF, or alternatively, that λ uses either IHF or another protein(s) in the encapsidation process. If the latter is true, the 22-kd protein identified in vitro might be the alternative to IHF in the *cos* cleavage reaction in vivo. A role for IHF in packaging is clearly indicated by studies with the lambdoid phage 21. Unlike λ, this phage does not grow lytically on *himA* or *hip* mutants and requires IHF for packaging in vitro (M. Feiss, S. Frackman, and J. Sippy, personal communication). In this work, we characterize a mutant of λ, λ *cos*154, whose properties suggest that IHF participates in DNA packaging in vivo.

MATERIALS AND METHODS

Bacterial and phage strains and media. The bacterial and phage strains used are shown in Table 1. No difference was observed in the efficiency of plating (EOP) of any phage between C600 and N99. The strain used in the red plaque test (5) to assay for Int activity was RW842. Single and multiple λ lysogens (λ *cos*154 *c*I857 or λ*cos*$^+$ *c*I857) in N99 were constructed as described previously (13, 18). The Δ*himA82* mutation was introduced into these strains by P1 transduction (24).

The λ *cos*154 *int*6 *c*I857 phage was obtained from the National Institutes of Health phage collection. It had been treated previously with nitrosoguanidine to generate the *int*6 mutation (17). The strains used for phage constructions (DC7, SA297, and N4830) are represented in Fig. 2. The λ *cos*154 *int*$^+$ *c*I857 phage was made by infecting SA297 with λ *cos*154 *int*6 *c*I857 and selecting for *int*$^+$ recombinant phage

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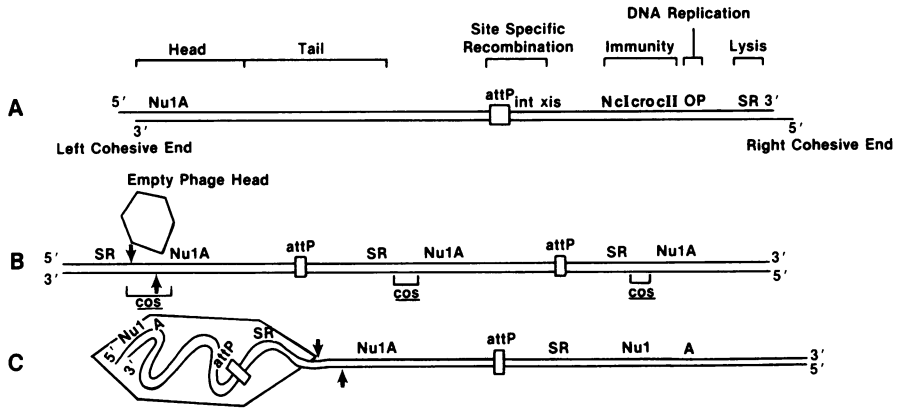

FIG. 1. A, The 48.5-kilobase linear λ DNA molecule. The λ cohesive ends are a 12-bp 5′ extension. The genome can be divided into areas involved in head and tail protein synthesis, site-specific recombination (integration and excision), immunity, DNA replication, and cell lysis. Selected genetic markers, including the leftmost genes *Nu*1 and *A* and the rightmost genes *S* and *R*, are indicated. B, The λ genomic concatemer produced by rolling circle replication of the circularized DNA. To package a unit length of λ DNA, the phage-encoded terminase protein binds to the λ DNA at *cos* and to the empty phage head. Single-strand nicks separated by 12 bp are introduced into the DNA at *cos* by terminase (vertical arrows). C, The left end of the λ DNA (indicated by the genes *Nu*1 and *A*) enters the phage head first. Terminase cleaves the DNA again upon encountering a second *cos* site. The cohesive ends are thus generated, and a unit length of λ DNA is encapsidated.

by the red plaque test with RW842. This λ *cos*154 *int*$^+$ *c*I857 phage exhibited the same phenotype in IHF-defective (IHF$^-$) *E. coli* as its *int*6 parent. The λ *cos*154 *int*6 *imm*^{21} phage was made by infecting DC7 with λ *cos*154 *int*6 *c*I857 and selecting for *imm*^{21} by plating on N4830.

The medium was Luria broth containing 1% Difco tryptone broth, 0.5% NaCl, and 0.5% yeast extract. Tryptone broth agar plates contained 1% Difco tryptone, 0.5% NaCl, and 1% agar. Phage were diluted in 10 mM Tris (pH 7.4), 10 mM $MgSO_4$, and 0.01% gelatin. Tryptone broth top agar contained 0.7% agar. When necessary, ampicillin was added to a final concentration of 50 μg/ml. Indicator agar plates for the red plaque test were tryptone broth with 1% galactose and 2,3,5-triphenyl-tetrazolium chloride as dye indicator (5).

Bacteriophage crosses. C600 cells were grown to 2×10^8/ml in Luria broth supplemented with 0.2% maltose. The cells were centrifuged and concentrated 5× in 10 mM $MgSO_4$. Cells (5×10^7) were infected with phage at a multiplicity of 3. After 10 min of adsorption at room temperature, the mix of phage and cells (50 μl) was exposed to UV irradiation (300 ergs per mm^2). Luria broth (10 ml) with 5 mM $CaCl_2$ was added, and the infections were incubated with shaking at 38°C for 90 min. After the addition of chloroform, the lysate was vortexed, and the debris was removed by centrifugation.

Marker rescue experiments. C600 was transformed to ampicillin resistance with either pBR322 vector DNA or pHC79 cosmid DNA, and N4830 was transformed to ampicillin resistance with pKC30*int* (D. Court, unpublished data) by standard procedures (38). Note that N4830 does not carry the wild-type allele of *int*6. The pKC30*int* plasmid carries the entire *int* gene. The phage infections were carried out as described above, with the exception that the multiplicity of phage infection was 0.5. The supernatant was removed to a fresh tube, diluted, and used to score for plating efficiency on K1942 (Δ*himA82 gyrB230*). To screen for *int*$^+$ recombinant phage, the diluted supernatant was used to infect RW842.

DNA sequencing studies. Phage DNA was treated with bacterial alkaline phosphatase to remove the 5′-phosphate (BAP · MATE; Bethesda Research Laboratories, Inc., Gaithersburg, Md.). These ends were then labeled with ^{32}P by using [γ-^{32}P]ATP and polynucleotide kinase. Alternately, the 3′ ends of the phage DNA were labeled with [α-^{32}P]dATP by using the Klenow fragment of *E. coli* DNA polymerase. An *Hpa*I restriction fragment containing the first 734 bp of the λ left-end terminus was isolated and purified. The modification and cleavage reactions of Maxam and Gilbert were used (25), and the resulting preparations of DNA were electrophoresed in 8% and 6% polyacrylamide gels. The gels

TABLE 1. Bacterial and phage strains

Strain	Description	Source or reference
Bacteria		
C600	*supE tonA thr leu thi*	NIH[a] strain collection
N99	*sup*^{0} *rpsL galK2*	NIH strain collection
K634	N99 *himA42*	(28)
E268	N99 *hip-157*	E. Flamm
JD12	N99 *himA42 hip-157*	K. Abremski
K1299	N99 Δ*himA82*	H. Miller (11)
E444	N99 Δ*hip1*	E. Flamm
K1870	N99 *gyrB230*	(12)
K807	N99 *gyrB114*	(29)
K1942	N99 Δ*himA82 gyrB230*	(11)
RW842	HfrH *galT*::(λΔ[*int*-FII])	(5)
K159	N99(λ *imm*^{434})	D. Friedman
K124	N99(λ *c*$^+$)	D. Friedman
Phage		
λ *cos*154 *int*6 *c*I857		NIH phage collection (18)
λ *cos*154 *int*$^+$ *c*I857		This work
λ *cos*154 *int*6 *imm*^{21}		This work
λ *int*6 *red*3 *imm*^{21} *c*		NIH phage collection
λ *imm*^{21} *c*		NIH phage collection
λ *c*I857		NIH phage collection
λ *imm*^{434}		NIH phage collection
λ *A*am11 *c*I857		NIH phage collection
λ *L*am63 *c*I857		NIH phage collection
λ *c*I857 *P*am3		NIH phage collection
λ *c*I857 *R*am5		NIH phage collection

[a] NIH, National Institutes of Health.

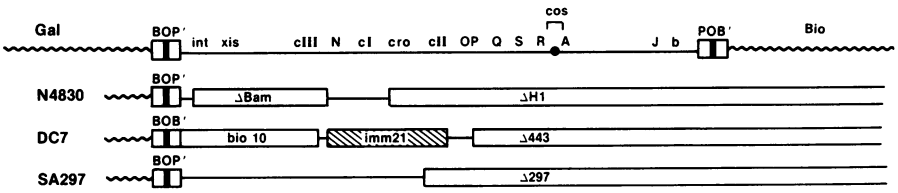

FIG. 2. Representation of an integrated λ prophage (top line) and the deletions or substitutions in strains used in this work. N4830 (M. Gottesman) is a λ *c*I857 lysogen with a deletion from the *Bam*HI site in the *int* gene to a *Bam*HI site near *c*III. Prophage deletion ΔH1 removes DNA from *cro* into the *bio* operon of *E. coli*, deleting all intervening genes of λ. Both DC7 and SA297 are F⁻ *rpsL his relA* (D. Court and S. Adhya, unpublished data). The prophage in DC7 is the result of the integration of an *imm*^{21} *bio* transducing phage at the λ attachment site. The Δ443 endpoint was determined to be within gene *O* (S. Adhya, personal communication). The endpoint of the prophage deletion in SA297 was mapped between *cro* and *c*II. The strain is Cro⁺, but CII⁻.

were autoradiographed, and the DNA sequence starting at the λ left cohesive end was determined.

RESULTS

Effect of the *cos*154 mutation on λ lytic growth in IHF⁻ hosts. The mutant phage was found in a λ strain that had undergone nitrosoguanidine mutagenesis (17) and had the characteristic of poor lytic growth on IHF⁻ *E. coli*. The phenotype of the mutant phage was demonstrated by the EOP of both λ *cos*⁺ and λ *cos*154 on a series of isogenic strains with mutations in IHF genes (Table 2). The *cos*154 phage forms tiny plaques on most of the IHF⁻ strains, and in the case of some IHF mutants shows a markedly reduced EOP.

IHF has been identified as a multimer of the *himA* and *hip* gene products (32, 36; E. Flamm, personal communication). *E. coli* with mutations in *himA* or *hip* are defective in λ site-specific recombination (27, 29), suggesting that each gene product does not function independently. In contrast, single point mutations in either *himA* or *hip* do not have a severe effect on the EOP of λ *cos*154 (Table 2). This also appears to be true for a *hip* deletion and to a lesser extent for a *himA* deletion. However, the λ *cos*154 exhibits an EOP that is 0.01% that of λ *cos*⁺ in *E. coli* with the double point mutations *himA42* and *hip-157*. These data suggest that either IHF subunit may function independently to support lytic growth of λ *cos*154.

Mutations in *gyrB* (*himB*) alter the β subunit of DNA gyrase and reduce λ DNA supercoiling. Because of this, λ site-specific recombination is reduced in *gyrB* mutants (11, 15). λ *cos*⁺ and λ *cos*154 were examined for their EOP on two strains each with a single *gyrB* mutation and on a strain with a *himA* deletion and a *gyrB* point mutation (K1942). λ *cos*154 forms normal-sized plaques on hosts with either the *gyrB230* or *gyrB114* mutation (Table 2). However, the EOP of λ *cos*154 on K1942 is 10% that of the EOP of λ *cos*154 on *E. coli* with a *himA* deletion. The fact that the *gyrB* mutations alone exhibit no observable effect on the growth of λ *cos*154 leads us to conclude that the *gyrB* mutation in K1942 amplifies the specific effect of the *himA* deletion. Recent findings indicate that the *gyrB* mutation decreases the burst of λ (12). This may be the effect of the *gyrB* mutation in K1942.

***cos*154, a *cis*-dominant mutation.** Does the mutation have an effect on a *cis*- or *trans*-acting λ component? This question was addressed by performing a dominance test. λ *cos*⁺ and λ *cos*154 were used to infect N99 and K1942 (*ΔhimA82 gyrB230*), each at a multiplicity of infection of 5. The resulting burst sizes are presented in Table 3. In N99, the burst size of each phage was comparable and not substantially altered upon mixed infection. In K1942, the burst size of λ *cos*⁺ was not lowered significantly, but that of λ *cos*154 was decreased and did not increase as a result of mixed infection. This indicates that the defect of λ *cos*154 is not in a *trans*-acting function and represents a mutation in a site on the λ chromosome or, less likely, a mutation in a *cis*-acting protein.

Genetic location of the *cos*154 mutation. Phage crosses were used to position the *cos*154 mutation to either the left or right of the λ immunity region (*imm*). A diagram of the crosses and the results are shown in Fig. 3. In cross A, λ *cos*154 *int*6 *imm*^{21} phage were crossed with either λ *imm*$^{\lambda}$ *P*am3 or λ*imm*$^{\lambda}$ *R*am5 phage. Recombinants *imm*$^{\lambda}$ am⁺, in which the crossover had occurred between *imm* and either gene *P* or gene *R* (represented by the hatched area), were selected by the ability to grow on DC7, a nonsuppressor strain immune to infection by phage 21. These recombinant phage were then screened for the *cos*154 mutation by testing for reduced EOP on K1942. No correlation was observed between the length of the crossover region (hatched area)

TABLE 2. EOP of λ *cos*154 on IHF⁻ *E. coli*

Strain	Relevant genotype	EOP of phage	
		λ *imm*^{21} *c*	λ *cos*154 *int*6 *imm*^{21}
N99	*him*⁺	1.0[a]	1.0
K634	*himA42*	0.8	0.7
E268	*hip-157*	0.5	0.5[b]
JD12	*himA42 hip-157*	0.1	10^{-4}
E444	*Δhip1*	0.9	0.6[b]
K1299	*ΔhimA82*	0.6	10^{-2}
K1870	*gyrB230*	1.0	0.8
K807	*gyrB114*	0.9	0.8
K1942	*ΔhimA82 gyrB230*	0.4	10^{-3}

[a] The titer of each phage on N99 at 38°C was given a value of 1. The EOPs on other strains are expressed as fractions of this number.
[b] Plaques were very small with ragged edges.

TABLE 3. Complementation studies with λ *cos*⁺ and λ *cos*154

Strain	Genotype	Burst of phage at 32°C[a]	
		λ *cos*⁺ *imm*^{434}	λ *cos*154 *c*I857
N99	*himA*⁺	67	
N99	*himA*⁺		54
N99	*himA*⁺	60	17
K1942	*ΔhimA82*	19	
K1942	*ΔhimA82*		1
K1942	*ΔhimA82*	32	1

[a] The burst sizes at 32°C after 120 min were measured by determining the titer of the phage on K159 (for burst of *imm*$^{\lambda}$ phage) and on K124 (for burst of *imm*^{434} phage). The burst was calculated as the phage titer divided by the number of infected bacteria.

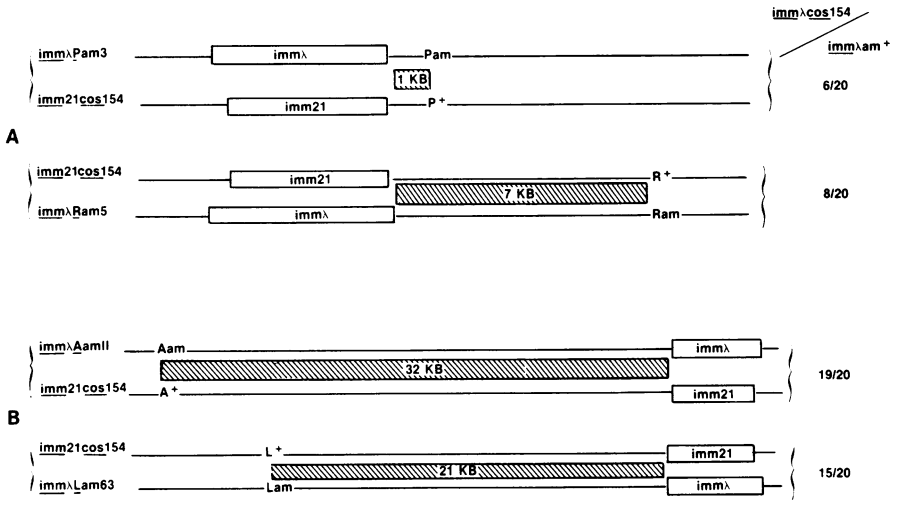

FIG. 3. Genetic mapping of λ *cos*154. (A) Representation of the right half of the phage genome in phage cross A. λ *cos*154 *int*6 *imm*^{21} was crossed with either λ*imm*$^{\lambda}$ *P*am3 or λ*imm*$^{\lambda}$ *R*am5. Twenty recombinant phage from each cross that were *imm*$^{\lambda}$ am^{+} were screened for the *cos*154 phenotype by examining the EOP on K1942. The number of *cos*154 phage is expressed as a fraction of the 20 *imm*$^{\lambda}$ am^{+} phage that were screened. (B) Representation of the left half of the phage genome in phage cross B., λ *cos*154 *int*6 *imm*^{21} was crossed with either λ *A*am11 *imm*$^{\lambda}$ or λ *L*am63 *imm*$^{\lambda}$. 20 recombinant *imm*$^{\lambda}$ am^{+} phage were screened for the *cos*154 phenotype by examining the EOP on K1942. In each case the hatched lines indicate the area (given in kilobases) in which homologous recombination can occur to yield the *imm*$^{\lambda}$ am^{+} recombinants.

and the frequency of λ *cos*154 recombinants. Thus, the *cos*154 mutation is most likely located to the left of the *imm* region. To test this inference, cross B was done. λ *cos*154 *int*6 *imm*^{21} phage were crossed with either λ*imm*$^{\lambda}$ *A*am11, or λ*imm*$^{\lambda}$ *L*am63 phage. Phage resulting from recombination events in either cross were selected as *imm*$^{\lambda}$ am^{+} by the ability to grow in DC7 as above. These recombinant phage were then screened for the *cos*154 phenotype by inability to grow lytically on K1942. A direct correlation was found between the length of the crossover region and the frequency of λ *cos*154 recombinants. The high percentage of recombinants with *cos*154 in both crosses indicates that the mutation is to the left of the amber markers used and more tightly linked to the *A* gene. The rarer *cos*$^{+}$ recombinants (1 of 20, 5 of 20) presumably resulted from a second crossover between the selected amber markers and the *cos*154 mutation. In cross A, double crossovers account for the rarer *cos*154 recombinants found in both cases.

The *A* gene is near the left terminus of the λ genome, the open reading frame beginning at bp +641 (4, 37). To position the *cos*154 site more exactly, the *cos*$^{+}$ allele was rescued from a cosmid recombinant molecule, pHC79 (20). This cosmid contains the wild-type λ *cos* region inserted into the plasmid vector pBR322. The first 415 bp from the left end of the λ genome are included in this DNA molecule. C600, transformed with either pHC79 DNA or pBR322 DNA (as a control), was infected with λ *cos*154 *int*6 *imm*^{21}. If the *cos*154 site is within the first 415 bp of the λ genome, then *cos*$^{+}$ phage could arise by homologous recombination with the cosmid, but not with the pBR322 plasmid. To detect the recombinants, phage resulting from the infection were plated on K1942. In two independent experiments, the EOP of phage recovered from C600 containing pHC79 was 10-fold higher than that of phage recovered from the infection of C600 containing pBR322, indicating rescue of the *cos*$^{+}$ site from the pHC79 DNA. To establish an independent measure of marker rescue frequency from a plasmid, N4830 cells transformed with a plasmid carrying the *int* gene (pKC30*int*, see above) were infected with λ *cos*154 *int*6 *imm*^{21}. A 10-fold increase in *int*$^{+}$ phage was detected after infection, similar to the level of rescue of *cos*$^{+}$ phage.

Molecular identification of the base changed by the *cos*154 mutation. The results of the genetic mapping experiments located the *cos*154 site close to the λ left end. To identify the mutation at the nucleotide level, we determined the DNA sequence of λ *cos*154 and the λ *cos*$^{+}$ recombinant phage generated in the λ *cos*154 *int*6 *imm*^{21} × λ *imm*$^{\lambda}$ *A*am11 phage cross (Fig. 3, cross B).

Polynucleotide kinase and the Klenow fragment of DNA polymerase were used to ^{32}P label the 5′ and 3′ ends of λ DNA respectively. An *Hpa*I restriction fragment that contains the first 734 bp of the λ left end was isolated and purified from each labeled preparation. The base specific modification and cleavage reactions of Maxam and Gilbert (25) were employed, and the first 350 bp of each strand of each phage was determined.

The DNA sequence of the λ *cos*$^{+}$ recombinant was identical to the published λ wild-type sequence (4, 37). Only one change in the sequence of nucleotides from the λ *cos*154 phage was detected. This is indicated in Fig. 4 at bp +154 as a GC-to-AT transition (hence the name *cos*154). The mutation is in a noncoding region upstream of the open reading frame of the leftmost λ gene, *Nu*1 (37; bp +185 in Fig. 4), in agreement with its *cis*-dominant phenotype. This change occurs within one of two 16-bp repeated sequences that comprise a larger palindromic region between bp +47 and bp +160 (Fig. 4, i and ii). Two variations of this repeated sequence are found closer to the *cos* region and are noted in Fig. 4 (iii and iv). It appears that a single transition event at bp +154 is responsible for the inability of the mutant phage to grow lytically in IHF^{-} *E. coli*.

Effect of *cos*154 on DNA encapsidation. The location of the mutation suggested that the *cos*154 mutation might be defective in some aspect of the DNA packaging reaction in IHF^{-} hosts. We examined λ DNA packaging using a biological assay, the Ter test (18, 34). The Ter test is based on the observation that a full-length λ genome can be packaged from tandem prophage in the *E. coli* chromosome after

infection with an *int*$^-$ heteroimmune phage. Under these conditions, the prophage remain repressed and nonreplicating. The heteroimmune infecting phage provides the products necessary for efficient cleavage and packaging of the λ genome between the two *cos* sites in the repressed tandem prophage. In the case of a single prophage, packaging from the single *cos* site cannot encapsidate a complete λ genome. In our experiments, tandem multiple prophage of either λ *cos*$^+$ *c*I857 or λ *cos*154 *c*I857 were established in N99. The Δ*himA82* deletion was introduced into these lysogens by P1 transduction. If *cos*154 interferes with packaging in IHF$^-$ hosts, the yield of this prophage compared with the *cos*$^+$ prophage should be reduced.

The results of the Ter test with λ *int*6 *red*3 *imm*^{21} *c* as the heteroimmune infecting phage are shown in Table 4. In the *himA*$^+$ strain, no difference is found between the *cos*$^+$ and *cos*154 prophage yields. In the Δ*himA82* strain, the number of λ *cos*$^+$ *c*I857 phage from the multiple lysogen is not significantly reduced. However, in the Δ*himA82* strain, the prophage yield from the λ *cos*154 *c*I857 multiple lysogen decreases to 10% of that from the *himA*$^+$ strain. Moreover, it is 5% of that from the λ *cos*$^+$ *c*I857 multiple lysogen. We conclude from these data that λ *cos*154 is dependent upon IHF for efficient packaging of its DNA.

Revertants of λ *cos*154. The plaques that arise at a frequency of 10^{-4} on the *himA hip* double-point mutant (Table 2) or at a frequency of 10^{-3} on K1942 (Table 2) are of normal size. However, the high frequency suggests that they do not represent true revertants.

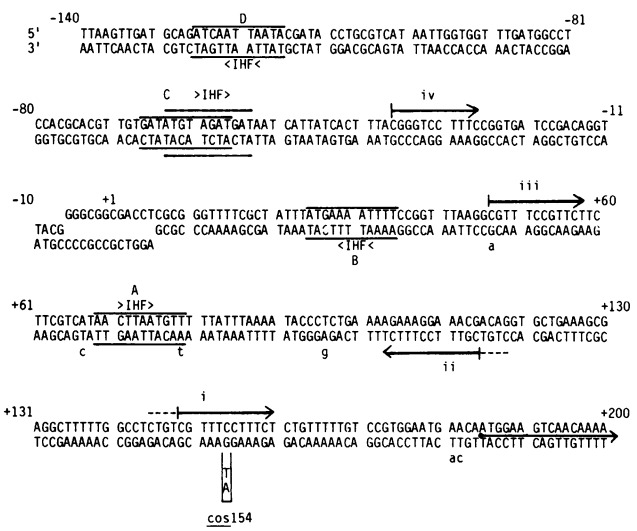

FIG. 4. λ *cos*$^+$ DNA sequence surrounding the junction of the cohesive ends from bp −140 to bp +200. The first bp to the right of the center of the 12 bp cohesive end junction is referred to as bp +1. This DNA sequence is identical to the published λ sequence (37). The DNA sequence of the first 350 bp of the left end of λ*cos*154 was determined. A single GC-to-AT transition was detected at bp +154. This mutation occurs in one of two 16-bp inverted repeat sequences between bp +102 and bp +160 (i and ii). A shorter 12-bp derivative of this sequence between bp +47 and bp +58 is also marked (iii). A less conserved 11-bp derivative from bp −37 to bp −27 can be seen as well (iv). Potential IHF binding sites are also indicated (A through D). The IHF concensus binding site is 5′ AANNNNTTG $\frac{A}{T}$ T 3′ (N. Craig and H. Nash, personal communication). Two potential sites, each with 2 bp changes, are noted at bp −54 and bp −57 (C). Two other derivatives of the IHF binding sequence, each with 1 bp change, are marked at bp +25 and bp +69 (B and A). The small letters indicate the additional changes detected when the sequence of the bottom strand of a *cos*154 revertant phage was determined. The start of the open reading frame for *Nu1* is bp +185 according to this system of bp numbering.

TABLE 4. Effect of *cos*154 on λ DNA packaging—results of the Ter test

Relevant host genotype	Prophage[a]	Prophage yield per bacterium
himA$^+$	(λ *c*I857)$_n$	1.25
himA$^+$	(λ *cos*154 *c*I857)$_n$	0.47
Δ*himA82*	(λ *c*I857)$_n$	0.72
Δ*himA82*	(λ *cos*154 *c*I857)$_n$	0.04
Δ*himA82*	(λ *cos*154 *c*I857)$_1$	0.0006

[a] Multiple lysogens are indicated with the subscript n. A yield of 1 from a λ *cos*$^+$ *c*I857 tandem double prophage in *himA*$^+$ *E. coli* is expected. The prophage yield from a single lysogen is shown for the Δ*himA82* host.

Four spontaneous "revertants" of λ *cos*154 *int*6 *imm*^{21} were isolated from single plaques arising on K1942. These phage form plaques with equal efficiency on N99 and K1942. Each phage was crossed with λ *cos*$^+$ *A*am11 *imm*$^\lambda$ (as in Fig. 3, cross B). Twenty-four *imm*$^\lambda$ am$^+$ phage were isolated from each cross (growth on a *sup*^{0} λ*imm*^{21} lysogen, DC7) and were examined for the *cos*$^+$ phenotype. All recombinant phage were *cos*$^+$, indicating a tight linkage between the site of "reversion" in each phage and gene *A*.

The DNA from one *cos*$^+$ "revertant" phage was isolated, and the 3′ ends were ^{32}P labeled with the Klenow fragment of DNA polymerase. An *Hpa*I restriction fragment of the first 734 bp of the λ left end was isolated and subjected to DNA sequence analysis as described above. The DNA sequence from bp +13 to bp +226 was determined. The DNA from the phage contained the *cos*$^+$ sequence (GC at bp +154). In addition, it contained four transitions and two transversions, noted by small letters at the appropriate location in Fig. 4. These numerous differences suggest that the DNA sequence of this phage derives from recombination with a cryptic prophage, and this recombination accounts for the high "reversion frequency" of λ *cos*154 (Table 2). Other examples of recombinational rescue of cryptic *cos* sites in *E. coli* have been reported (10).

DISCUSSION

We have characterized a mutant of λ, λ *cos*154, that exhibits reduced efficiency of plating on *E. coli* mutants defective in a DNA-binding protein, IHF. This *cis*-dominant mutation was genetically positioned close to the left cohesive end of the λ genome. DNA sequence determination revealed a single GC-to-AT transition event at bp +154 in a noncoding region between the λ left cohesive end and the first λ gene, *Nu*1. The mutation results in a defect in the in vivo packaging of λ *cos*154 DNA in IHF$^-$ *E. coli*. Successful packaging of λ DNA is the result of multistep processes requiring an initial cleavage at the *cos* site by λ-encoded terminase and subsequent uptake of the DNA into an empty phage head. The *cos*154 mutation may cause a defect in either or both of these processes.

Factors involved in *cos* cleavage. The terminase protein (the product of the phage *Nu*1 and *A* genes) binds and cleaves the phage DNA at *cos* (9). In vitro this reaction also requires an *E. coli*-encoded protein(s) (2, 16). The requirement for *E. coli* accessory factors can be satisfied either by a 22-kd protein or by IHF, an *E. coli* protein initially identified and purified as a host factor required for λ site-specific recombination. (The separate identity of these two proteins has not

been confirmed.) Our observation that IHF is not required for packaging of λ DNA in vivo, but is necessary when the phage carries thew *cos*154 mutation, is consistent with two models. (i) Host proteins are essential for DNA packaging. However, either the 22-kd protein or IHF can be used in the packaging reaction in vivo and in vitro. (ii) Bacterial proteins are not necessary for the packaging reaction. In vivo, terminase alone may be sufficient for the entire reaction, whereas in vitro, host proteins are required because conditions for packaging may be suboptimal.

In considering the role of the site defined by the *cos*154 mutation in the packaging reactions, it is important to keep in mind the position of this mutation: a transition 154 bp to the right of the center of the *cos* site. Significantly, the region of hyphenated dyad symmetry identified by *cos*154 is outside the boundaries of the minimum substrate required for in vivo and in vitro *cos* cleavage and DNA packaging (9, 19, 33). These boundaries extend from bp positions −40 to +120 of the *cos* region (Fig. 4). However, the experiments that have defined these boundaries utilize assay systems that contain IHF in vivo and in vitro. Therefore, it is not known whether terminase can cleave and package the minimum substrate DNA in the absence of IHF.

Role for *E. coli* proteins in DNA packaging. If an *E. coli*-encoded protein(s) is required for packaging in vivo, our experiments with λ *cos*154 demonstrate that IHF is a likely candidate. However, the fact that λ *cos*$^+$ phage grow lytically in cells that cannot supply IHF suggests that another protein can satisfy this requirement. An obvious candidate is the 22-kd protein that promotes *cos* cleavage in vitro. According to this idea, λ *cos*154 fails to grow in IHF mutants because the *cos*154 mutation alters the binding site for the 22-kd protein. Thus, IHF is required to support the terminase reaction in the presence of the *cos*154 mutation.

If the 16-bp sequence altered by *cos*154 is required for recognition by the 22-kd protein, then we suggest that the minimum substrate, which lacks this site, also requires IHF for efficient DNA packaging. Additionally, we predict that the minimum substrate contains IHF-binding sites. Examination of the nucleotide sequence reveals that the DNA of the minimum substrate includes two potential IHF-binding sites (Fig. 4; sites A and B) each 1 bp different from the consensus sequence derived by Craig and Nash (personal communication). Moreover, deletions of the minimum substrate that remove IHF site A decrease packaging efficiency by a factor of 10 (19).

Alternative role of the *cos*154 site. Examination of the region flanking the *cos* cleavage site reveals numerous repeated sequences (33) (Fig. 4). In fact, the 16-bp sequence altered by the *cos*154 mutation is repeated in inverted orientation 26 bp to the left (Fig. 4, ii). A derivative of this sequence is found closer to *cos* between the two potential IHF-binding sites (Fig. 4, iii between A and B). Another related sequence less conserved is located to the left of the *cos* cleavage site (Fig. 4, iv). In the absence of direct evidence for an *E. coli* protein interacting at these repeated sites, it is possible that they are instead the binding site for terminase itself. The results of DNA footprinting experiments (6) and substrate binding competition experiments (9) have demonstrated that terminase binds and protects this general region.

We suggested above that IHF may be required for *cos* cleavage or packaging (or both) only under conditions in which terminase interactions are weakened (a possible effect of the *cos*154 mutation). In these cases, IHF might be required to strengthen these interactions. A relationship between terminase and IHF is suggested from studies of the lambdoid phage 21. Phage 21 requires IHF for DNA packaging in vitro and in vivo (Feiss, Frackman, and Sippy, personal communication). This phenotype resembles that of the λ *cos*154 mutant and may be explained by the model that IHF strengthens a weaker phage 21-terminase interaction. In fact, *her* mutants of phage 21 that revert this phenotype and are packaged in IHF$^-$ hosts have alterations in gene *1*. This gene is analogous to *Nu*1 and encodes a subunit of phage 21 terminase (Feiss, Frackman, and Sippy, personal communication).

A model that proposes the *cos*154 site to be the terminase-binding site does not exclude the possibility of a function for the 22-kd protein isolated by Gold and Becker (16). It may also function during *cos* cleavage, but may be unable to support the packaging of the *cos*154 mutant DNA in the absence of IHF. Other repeated DNA sequences found in this region (33) may represent interaction sites of this protein.

Substrate similarity between *cos* and *att*. The evidence shows that IHF is involved in reactions that require a site-specific cleaveage of λ DNA, i.e., integrative and excisive recombination at the phage attachment site (*att*) and the packaging reaction at *cos*. These events have similar properties. In both, a phage-encoded protein binds and nicks the DNA; Int binds and cleaves the DNA at *att* (3), and terminase binds and cleaves the DNA at *cos* (9). The genes encoding these proteins are located adjacent to the site with which they interact (Fig. 1).

A comparison of the substrates used in these reactions reveals similarities. Both contain a core region in which the nicking reactions occur. In the DNA surrounding the core of *att*, IHF-binding sites are interspersed among binding regions for Int (reviewed in reference 41). Outside of the *cos* core region, potential IHF-binding sites are situated among proposed terminase interaction domains (Fig. 4, A, B, C, and D and i, ii, iii, and iv). The recognition sites for these DNA-binding proteins are located in both arms surrounding the *att* (41) and *cos* cores (Fig. 4). Int also binds to the core of *att*, but the binding is weaker and the sequence is different than in the sites surrounding the core (reviewed in reference 41). At the *cos* core, terminase presumably binds to cleave the DNA. This recognition sequence for terminase is different from the binding sequence outside the core (7, 9, 33).

IHF is an accessory protein in λ site-specific recombination. It has no demonstrated cleavage activity, but binds to specific sequences near the recombination site (N. Craig and H. Nash, personal communication) and promotes the binding of the λ Int protein nearby (36). We suggested above that IHF may also strengthen terminase binding in the *cos* region. Terminase protein from wild-type λ may be very efficient and not require the enhancing effect of IHF. Lambda site-specific recombination and phage 21 DNA packaging both require IHF, but an independence of IHF can be demonstrated in these systems as well. *int* mutants have been isolated that are independent of IHF for site-specific recombination in vivo, e.g., *int*-h3 (31). Analogous terminase mutants (*her*) of phage 21 do not require IHF for packaging. Therefore the possibility exists to isolate terminase mutants in the λ *cos*154 background that will be independent of IHF for DNA packaging.

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