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A point mutation in the *Nul* gene of bacteriophage λ facilitates phage growth in *Escherichia coli* with *himA* and *gyrB* mutations

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Summary. A mutant of λ was isolated that grows in the *Escherichia coli himAΔ/gyrB-him320*(Ts) double mutant at 42° C; conditions which are non-permissive for wild-type λ growth. The responsible mutation, *ohm1*, alters the 40th codon of the *Nul* reading frame. The *Nul* and *A* gene products comprise the terminase protein which cleaves concatameric DNA into unit-length phage genomes during DNA packaging. The *Nul-ohm1* gene product acts in *trans* to support λ growth in the double *himA/gyrB* mutant, and *λcos154* growth in the single *himA* mutant. The observation that an alteration in *Nul* suppresses the inhibition of growth in the double *himA/gyrB* mutant implicates DNA gyrase, as well as integration host factor, in the DNA:protein interactions that occur at the initiation of packaging.

Key words: *cos* – DNA gyrase – DNA packaging – Integration host factor – Terminase

Introduction

Packaging of phage λ DNA into proheads requires the action of terminase, an enzyme encoded by the phage *Nul* and *A* genes (Sumner-Smith et al. 1981), to cleave concatameric DNA into unit-length molecules with cohesive ends (*cos* sites). Following infection, the linear DNA circularizes by hydrogen bonding at *cos* (reviewed in Feiss and Becker 1983; Feiss 1986).

Terminase is thought to act at two sites within the *cos* region, initially binding to the phage DNA at *cosB* and, subsequently, nicking the DNA at *cosN* (Feiss et al. 1983). These reactions probably occur as the DNA is translocated into the prohead. When the substrate is concatameric λ DNA, terminase acts processively; one active *cosB* site will catalyze the packaging of tandem genomes even if subsequent sites lack functional *cosB*s (Feiss and Widner 1982).

Two host-encoded factors have been observed to enhance the activity of terminase in vitro (Gold and Parris 1986): terminase host factor (THF) and integration host factor (IHF). THF is a 22 kDa protein of uncharacterized genetic origin. IHF is a heterodimer composed of the products of the *himA* (IHFα; Miller and Friedman 1977; Williams et al. 1977) and *hip/himD* (IHFβ; Kikuchi et al. 1985) genes. IHF was identified because of its role as an accessory factor for the site-specific recombination reactions that result in integration and excision of the λ genome from the *Escherichia coli* chromosome (reviewed by Nash 1981).

Many phages with mutations in *cosB* are absolutely dependent on IHF for growth. In this study we use *λcos154* (Bear et al. 1984), a *cosB* point mutation located in one of 4 repeats of a degenerate 16 bp sequence (Fig. 1). Other phages with increased IHF-dependence are *λcos59* (Miller and Feiss 1988) and λ-21*hy*19, which contains the intrinsically IHF-dependent analogues of *cosB* and *Nul* from phage 21 (Feiss et al. 1985a).

Terminase binds more tightly in vitro when the substrate DNA is supercoiled (Feiss et al. 1983). DNA gyrase, the enzyme that catalyzes the supercoiling of DNA in vivo, is a heterodimer composed of the products of the *gyrA* and *gyrB* genes (reviewed in Gellert 1981). The λ int system has been shown to require supercoiled DNA as a substrate in vitro (Richet et al. 1986) and to be inhibited by specific antagonists of DNA gyrase in vivo (Kikuchi and Nash 1979; Abremski and Gottesman 1979). Indeed, mutations in *gyrB* have been characterized that block the integrative site-specific recombination of λ and are thus called *himB* (Miller et al. 1979). The most extensively studied of these mutations confers a Ts Him phenotype (site-specific recombination occurs at low, but not at high, temperatures); hence, the name, *gyrB-him*(Ts) (Friedman et al. 1984b). Double *himAΔ/gyrB-him*(Ts) mutants exhibit phenotypes which are not observed with the single mutants; e.g., λ fails to grow in the double mutant at 42° C, but grows under the same conditions in the single mutants (Friedman et al. 1984a).

In order to assess the nature of the block on λ growth in the double mutant, we isolated mutants of λ that form plaques on the *himAΔ82/gyrB-him230*(TS) strain. We report the characterization of one mutant that contains a point mutation which compensates for this block in phage growth. The mutation alters the *Nul* gene, resulting in a host factor-independent terminase.

Materials and methods

Strains and media. A list of all bacterial and bacteriophage strains used in this study is given in Table 1. Media for propagation of bacteria and phage have been described (Miller and Friedman 1980; Maniatis et al. 1982).

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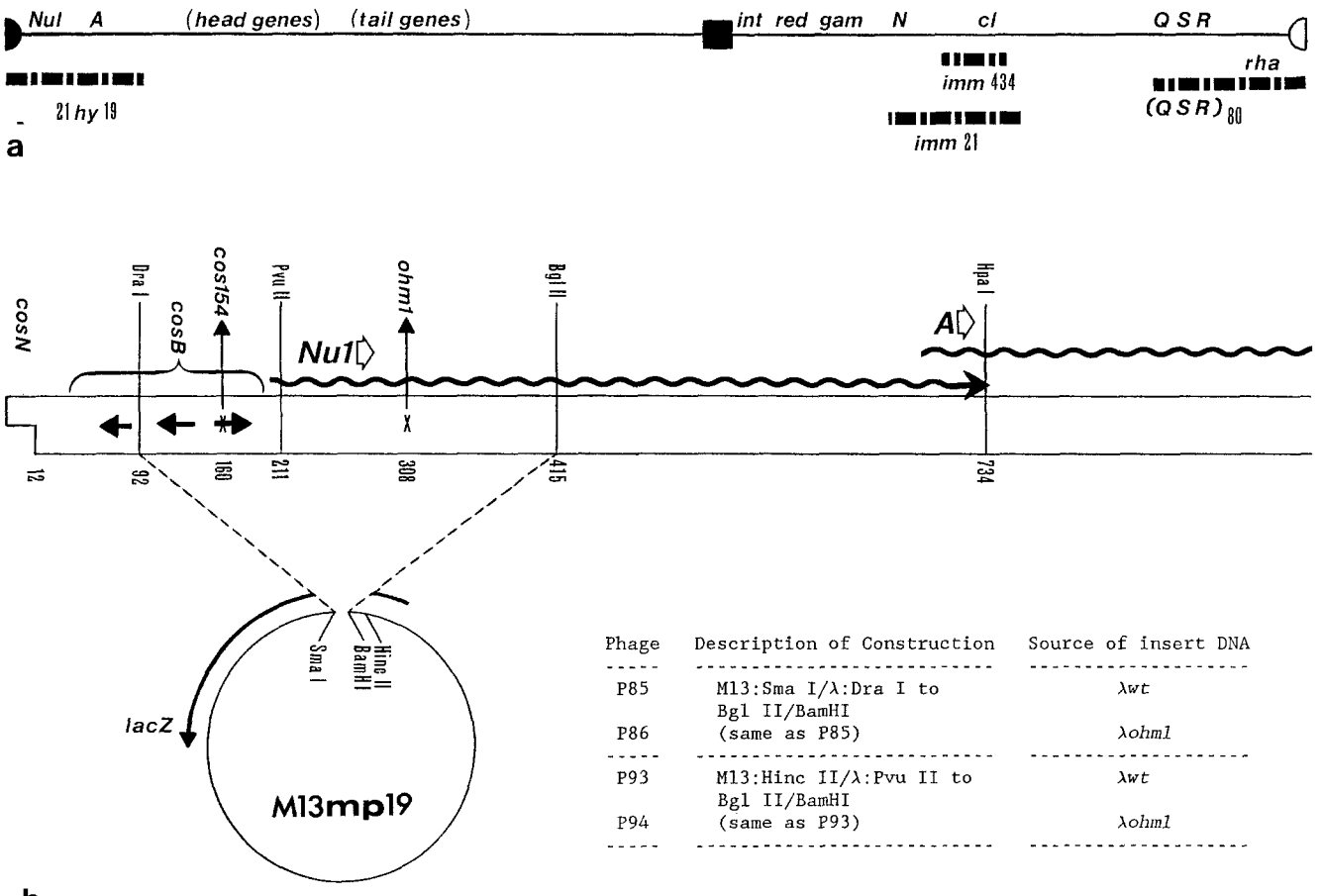


Phage	Description of Construction	Source of insert DNA
P85	M13:Sma I/λ:Dra I to Bgl II/BamHI	λwt
P86	(same as P85)	λohm1
P93	M13:Hinc II/λ:Pvu II to Bgl II/BamHI	λwt
P94	(same as P93)	λohm1

Fig. 1a, b. Subcloning of λ DNA into M13mp19 for marker rescue and sequencing. **a** An abbreviated map of the λ genome. The *square* near the middle of the map represents the *att* site; the *half-circles* locate the cohesive ends (*cos* sites). Positions of the genes discussed in the text are shown (*above*), as well as the location and extent of four substitutions which result in the replacement of λ DNA with that of related lambdoid phages (*below*). **b** A detailed map of the left-most 1 kb of the λ genome and the subcloning scheme used. The indicated fragment of λ DNA includes *cosN*, *cosB*, the complete *Nul* gene and the N-terminal portion of the *A* gene. The placement of the *bracket* corresponding to *cosB* is not meant to imply any functional limits to *cosB* (sequences on the other side of *cosN* may be involved as well). Two mutations used in this study are indicated by X. The *cos154* mutation lies at bp 160, in the most *Nul*-proximal copy of a repeated sequence indicated by *arrows*. The *ohm1* mutation lies in the *Nul* gene at bp 308. Restriction enzyme sites are listed *above*, their precise location *below*, the λ fragment map. Several of these sites were used in the M13 constructions described in the Table at the bottom of the figure. The *dashed lines* show the cloning scheme used to construct P85 and P86; the constructions P93 and P94 are described, but are not diagrammed. *Note*: Attempts to subclone the *Dra*I – *Bgl*II fragment of λ into M13mp18 failed repeatedly. The DNA sequence of the hypothetical subclone predicts that the *Nul* gene would be fused, in frame, with the *lacZ* gene of the vector. It is possible that production of this fusion protein is lethal to the vector or host

Genetic techniques. Phage bursts were performed as in Friedman et al. (1984b), e.g., log-phase cells (genotypes are indicated in the Tables) were infected with the various phages at a multiplicity of infection (moi) of 0.1 and incubated at the indicated temperatures for the indicated times. Adsorptions were carried out at 42° C when the experiment included strains carrying the *gyrB-him230*(Ts) mutation. Complementation studies were performed as in Mozola and Friedman (1985): log-phase cells were infected with phages at a moi of 5 for each phage and incubated at the given temperatures for the given times. Quantitative Ter tests were performed as in Friedman and Yarmolinsky (1972), with the dilysogens being infected with phage at a moi of 5 and incubated at 32° c for two h. In these three procedures, the infected cells were treated with chloroform and pelleted before being titered on K37 (bursts), K124 and K159 (complementation studies), or K455 and K2850 (Ter tests).

Marker rescue from cloned DNA was as follows: A single plaque of an M13mp19 clone was added to 8 ml of 2 × YT broth with 40 µl of a fresh JM101 starved lawn. After incubation at 37° C for 4 h, 330 µl were removed, added to 1×10^9 of the appropriate λ phage and kept at 25° C for 20 min to facilitate adsorption of the λ phage. The mix was then added to 7.5 ml LB broth containing 0.2% maltose and 10 mM $MgSO_4$ and incubated with aeration at 37° C for 2 h. The samples were treated with chloroform, centrifuged to remove bacterial debris and titered on the selective and non-selective strains.

Construction of himAΔSma

(1) *Deletion of plasmid DNA.* Plasmid pHIMA4 (gift of H. Miller) DNA was digested with *Sma*I restriction endonuclease, diluted and ligated. Deletion derivatives were identified as the plasmids in *himA*$^-$ transformants that fail to

Table 1. Strains

Strain	Relevant markers	Source/reference[a]
A. Escherichia coli		
K37(N99)	*sup°* *strA* *galK2*	M. Yarmolinsky
K124	K37 (*λwt*)	
K159	K37 (*λimm*434)	
K455	K37 (*λwt*) (*λimm*21)	
K936	K37 *himA42*	Miller and Friedman (1980)
K1299	K37 Tn*10-himAΔ82*	H. Miller (Miller 1984)
K1617	W3350 *mutD5*	E. Flamm (Fowler et al. 1974)
K1870	K37 *gyrB-him230*(Ts)	Friedman et al. (1984b)
K1942	K37 Tn*10-himAΔ82* *gyrB-him230*(Ts)	Friedman et al. (1984a)
K2691	K37 *himAΔSma*	This work
K2792	K37 *himAΔSma* *gyrB-him230*(Ts)	This work
K2850	K37 (*λimm*434) (*λimm*P22)[b]	
K2856	K37 (*λcI857cos154*) (*λimm*434)	
K2858	K37 (*λimm*434) (*λcI857cos154*)	
K3079	K2856 Tn*10-himAΔ82*	
K3080	K2858 Tn*10-himAΔ82*	
JM101		W. Dunnick

Phage	Source/reference
B. Bacteriophages	
λwt (*λpapa*)	M. Yarmolinsky
λc160	M. Gottesman
λcI857	M. Yarmolinsky
λcI857cos154	D. Court (Bear et al. 1984)
λohm1	This work
$\lambda(QSR)_{80}$	(Mozola and Friedman 1985)
λimm21c⁻int6red3	M. Yarmolinsky
λimm21c⁻int6red3ohm1	This work
λimm434cI2	M. Yarmolinsky
λimm434cI12ohm1	This work
λimm434cI2cos154	(Bear et al. 1984)
λimm434cI2 λ-21hy19	M. Feiss (Feiss et al. 1985a)
Muc*25*	A. Bukhari

[a] Strains with no source listing were constructed in this laboratory
[b] Lysogens of *imm*P22 phage will exclude phage 21, as these phage have identical repressor genes

support growth of IHF-dependent phages (i.e., mu, *λrha*, *λcos154*, etc.). Based on the published sequence (Miller 1984), deleting the DNA between the *Sma*I sites results in the loss of a portion of the *himA* gene and shifts the remaining *himA* reading frame, beginning at codon number 61, so that translation terminates at what would otherwise be codon number 65. (The native reading frame is 99 codons in length.) This altered *himA* gene is named *himAΔSma*.

(2) *Crossing himAΔSma onto the chromosome.* The *Sma*I-deleted plasmid (pHIMA4Δ*Sma*) was transformed into the *himA*$^+$ strain K37. Bacteria were grown to log phase in LB, sedimented, resuspended in phosphate buffer (70 mM NaCl, 50 mM $Na_2HPO_4 \cdot 7H_2O$, 20 mM KH_2PO_4, pH 6.0) and UV irradiated at 12 ergs/mm^2 for 25 s. Bacteria were grown overnight in LB broth, and homogenatized derivatives (in which the *himAΔSma* mutation was crossed onto the chromosome) were then isolated by counterselection with Muc*25* phage. One derivative was purified and cured of plasmid to generate K2691.

Nucleic acid techniques and enzymes. Phage DNA was purified by the procedure described by Arber et al. (1983). Plasmid preparations, restriction digests, ligations and transformations were performed essentially as described by Maniatis et al. (1982). Enzymes were from New England Biolabs, except for *E. coli* DNA polymerase I large fragment (Bethesda Research Laboratories).

Sequencing. Sequencing was carried our according to the chain-terminating method of Sanger et al. (1977) with the modifications given in Schauer et al. (1987).

Results

Isolation of λohm1

Derivatives of *λ* capable of growing in a *himAΔ82/gyrB-him230*(Ts) strain (K1942) were isolated from a lysate of *λwt* that had been mutagenized by growth in the *E. coli* mutator strain K1617 (Fowler et al. 1974). The mutated lysate yielded plaques on a lawn of K1942 at 42° C at a frequency of 10^{-6} per viable phage particle. One plaque was chosen for further study and the phage was named *λohm1*.

Growth of λohm1 *in* E. coli himAΔSma/gyrB-him230*(Ts)*

The ability of *ohm1* to influence *λ* growth was assessed using burst size as a measure of phage growth (Table 2). A *λohm1* phage shows a consistent fivefold larger burst

Table 2. Growth characteristics of *λwt* and *λohm1* in *Escherichia coli himAΔSma/gyrB-him230*(Ts) (K2792)

Phage[a]		Burst of phage
A. Bursts (42°C, 120 min)		
λwt		6.2
λohm1		33
B. Complementation studies (42° C, 90 min)		
λwt		3.0
*λimm*434		2.6
*λimm*434*ohm1*		17
λwt		1.3
*λimm*434	+	0.6
λwt		7.1
*λimm*434*ohm1*	+	8.7

[a] All phages used were *cI⁻*

Table 3. Influence of integration host factor on growth characteristics of *λwt*, *λohm1* and *λcos154*

Phage[a]	Burst in K37 (*himA*$^+$)	Burst in K2691 (*himAΔSma*)	Relative burst[b]
A. Bursts (37° C, 120 min)			
λwt	760	190	0.25
λcos154	580	15	0.03
λohm1	740	590	0.83

B. Complementation studies in K2691 (*himAΔSma*) (37° C, 90 min)

Phage(s)		Burst of phage
λcos154		4.7
*λimm*434		39
*λimm*434*ohm1*		160
λcos154 / *λimm*434	+	8.6 / 33
λcos154 / *λimm*434*ohm1*	+	30 / 130

[a] All *λ* derivatives were *cI857*; all *imm*434 derivatives were *cI*$^-$
[b] The relative burst is the burst in K2691 divided by the burst in K37

in the double *him* mutant at 42° C in comparison with the parent phage. However, this burst is lower than that observed for *λwt* in a *him*$^+$ bacterium (compare with Table 3A).

The complementation pattern of the *ohm1* mutation was examined using a mixed infection assay. As shown in Table 2B, when *λ* and *λohm1* coinfect a *himAΔSma/gyrB-him230*(Ts) host, the *ohm1* mutation is dominant. In comparison with single infections: (1) there is an increase in the burst of the wild-type (*ohm*$^+$) phage with a reduction in the burst of the *ohm1* phage, and (2) the total burst of the two phages is the same as that seen with the *ohm1* phage alone.

Complementation studies in E. coli himAΔSma

Although not resulting in a loss of plaque formation, single *him* mutants also influence *λ* growth. There is a slight, but consistent, reduction of the burst of *λ* in *E. coli himAΔSma* (*gyrB*$^+$). The *ohm1* mutation overcomes this defect, resulting in a burst of *λohm1* in a *himAΔSma* strain which is comparable to that of *λwt* in a *himA*$^+$ strain (Table 3A).

We next tested to see if *ohm1* could act in *trans* under *himA*$^-$ conditions. Since *λ* makes a burst, albeit smaller, in *himA* mutants, the complementation test was performed with a *λ* mutant, *λcos154*, which exhibits a more significant reduction of burst in *himA* mutants. The *cos154* mutation, located in *cosB*, is *cis*-acting and increases the dependence of terminase activity on IHF (Bear et al. 1984). As shown in Table 3B, *λohm1* complements *λcos154* for growth in the *himAΔSma* host. The burst of *λcos154*, when grown with *λohm1*, is comparable to the burst of *λwt* (*λimm*434 for this experiment) under the same conditions. Moreover, the total burst of *ohm1* and *cos154* phages is the same as seen with the singly-infecting *ohm1* phage. Since the burst of *ohm1* phage is greater than the burst of *cos154* phage in the double infection, the *ohm1* gene product may act preferentially in *cis*.

Table 4. Complementation studies of *λohm1* and *λ*(QSR)$_{80}$ in *Escherichia coli himA42* (K936)

Phages[a]		Burst of phage (32° C, 120 min)
λwt / *λimm*434	+	230 / 78
λ(QSR)$_{80}$ / *λimm*434	+	1.3 / 2.2
λwt / *λimm*434*ohm1*	+	130 / 170
λ(QSR)$_{80}$ / *λimm*434*ohm1*	+	1.7 / 4.3

[a] All phages used were *cI*$^-$

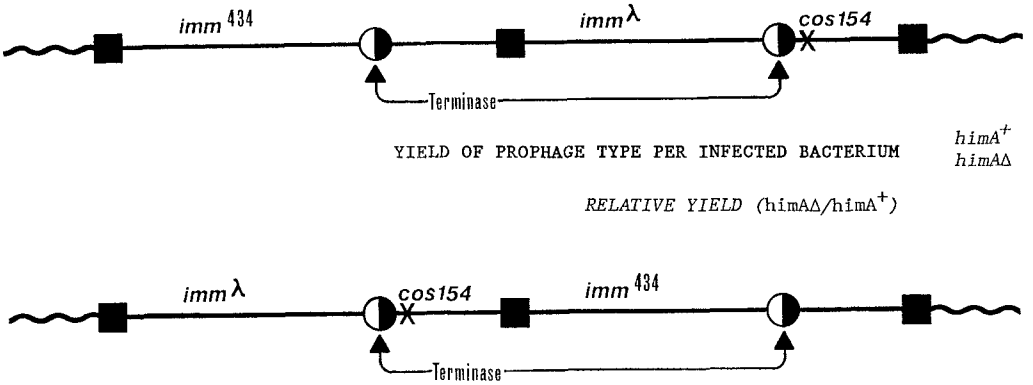
In order to determine if the suppression by *ohm1* is specific for terminase or is general for all phage-encoded variations that reduce growth in *himA* mutants, we tested the ability of *λohm1* to complement *λ*(QSR)$_{80}$, a phage that has a substitution from phage *φ*80 that includes *rha* which renders phage growth dependent on IHF (Mozola and Friedman 1985). Not only does *λohm1* fail to complement *λ*(QSR)$_{80}$ but, like *λwt*, *λohm1* is negatively complemented by *λ*(QSR)$_{80}$ in a *himA* mutant (Table 4).

Ter test

Because the complementation results might be explained on the basis of recombination of the two phage genomes and subsequent packaging from a mixed *ohm*$^+$/*ohm1* concatamer, the activity of terminase was assayed in vivo using the Ter test. This assay involves passive cleavage and encapsidation of a substrate of repressed tandem prophages (Gottesman and Yarmolinsky 1968; Mousset and Thomas 1969). The tandem prophages must have immunity repressor genes different from those of the infecting phage. The infecting phage is thus not repressed since it is heteroimmune to the prophages, and can express its functions. The prophages remain repressed and cannot replicate. Their combined DNA, however, contains two *cos* sites and is therefore an appropriate substrate for terminase, and other packaging functions, supplied in *trans* from the infecting phage. Because the prophage assumes a permuted order when integrated into the bacterial chromosome, cleavage at the *cos* sites in a double tandem lysogen releases one phage genome that is a hybrid of the two prophage genomes (see Fig. 2). Since the prophages remain repressed and cannot be replicated by functions from the superinfecting phage (Thomas and Bertani 1964), a priori it can be argued that, at most, there can be 1 (or 2, if host replication has proceeded through the prophage DNA) of the prophage type packaged and released per infected bacterium.

Encapsidation is a polarized reaction and only the *cosB* of the initial prophage (according to standard convention, the one on the left as shown schematically in Fig. 2) needs to be active in the assay (Feiss et al. 1985b). The constructions employed in these experiments have, in one case, the wild-type *cosB* placed in the functional position, while in the other case the *cosB* containing the *cos154* mutation is placed in this position.

When the substrate for packaging is *cos*$^+$ at the functional *cosB*, packaging by the wild-type (*ohm*$^+$) terminase in the *himAΔ82* dilysogen is reduced to 33% of that ob-



		Nul ALLELE OF SUPERINFECTING PHAGE[a] (Terminase supplier)	
		wt	*ohm1*
YIELD OF PROPHAGE TYPE PER INFECTED BACTERIUM	*himA*$^+$	1.8	1.2
	*himA*Δ	0.60	1.9
RELATIVE YIELD (himAΔ/himA$^+$)		*0.33*	*1.6*
YIELD OF PROPHAGE TYPE PER INFECTED BACTERIUM	*himA*$^+$	1.9	0.61
	*himA*Δ	0.17	0.53
RELATIVE YIELD (himAΔ/himA$^+$)		*0.09*	*0.87*

[a] The superinfecting phages are λ*imm*21*c*$^-$*int6red3*.

Fig. 2. Results of the Ter test. Tandem dilysogens of λ*cI857cos154* and λ*imm*434 were constructed with the two different prophage orders shown. Symbols are: ■, *att* sites; ◑, *cos* sites; —, prophage DNA; and ~, chromosomal DNA. The prophage genetic order differs from that in the standard genetic map. Terminase, provided in *trans* from a superinfecting phage, can act at the two *cos* sites, as indicated by the *lines with arrowheads*, leading to the packaging of a phage genome that is a hybrid of the two prophages. The phage supplying terminase (and other packaging functions) is λ*imm*21*c*$^-$*int6red3*; the *int6* mutation prevents the release of prophage sequences by the route of site-specific recombination. Dilysogens were constructed, and the prophage order confirmed, as previously described (Friedman and Yarmolinsky 1972). The *himAΔ82* mutation was introduced into the dilysogens by P1 transduction. In the dilysogen diagrammed above, the hybrid phage packaged from the chromosome has *imm*λ, the reverse-ordered dilysogen diagrammed below yields *imm*434 phage. Yields of the prophage types from these two dilysogens and derivative dilysogens that contain the *himAΔ82* mutation, during superinfection with λ*wt* or λ*ohm1*, are given on the right. The released prophages were identified as plaque-formers on K2850 (plates only *imm*λ phage; upper dilysogen) or K455 (plates only *imm*434 phage; lower dilysogen). The prophage yield is the number of phage of the indicated immunity divided by the number of infected bacteria

served in the *himA*$^+$ control. A substrate with the *cos154* mutation in the functional *cosB* shows a greater dependence on IHF; packaging by the wild-type terminase in a *himAΔ82* host is reduced to 9% of that observed in the *himA*$^+$ control.

Similar experiments using λ*ohm1* as the supplier of terminase showed that *ohm1* obviates the requirement for IHF in packaging. Packaging of *cos*$^+$ and *cos154* substrates in a *himAΔ82* strain by the *ohm1* terminase was, respectively, 160% and 87% of that seen in the isogenic *himA*$^+$ control.

Mapping of ohm1

The *ohm1* mutation was mapped to the far left side of the λ genome (according to the standard genetic map). Genetic crosses showed that *ohm1* was closely linked to the *Aam11* and *cos154* mutations (data not shown). Cloned fragments of λ DNA were employed in marker rescue experiments to locate precisely the *ohm1* mutation. The *ohm1* mutation could be rescued from either of two M13 subclones of λ*ohm1*, P86 or P94 (see Fig. 1), at a frequency of 2.9×10^{-5} and 1.9×10^{-5}, respectively, but not from M13mp19 alone or from analogous M13 clones containing wild-type λ DNA (P85, P93). In these experiments, recombinants with the *ohm1* mutation were selected as plaque-formers on a lawn of K2792 [*himAΔSma*/*gyrB-him230*(Ts)] incubated at 42° C; e.g., such recombinants were isolated following a cross of λ*cos154* with the plasmid form of an M13 construct (P94) containing a 204 bp fragment that had been subcloned from λ*ohm1*. Since this *Pvu*II – *Bgl*II fragment does not cover the site of *cos154*, the recombinant phage must be double *cos154-ohm1* mutants. Therefore, the *ohm1* mutation suppresses the effect of the *cos154* mutation.

Identification of the nucleotide change associated with ohm1

Employing the chain-termination method of DNA sequencing, we determined the sequence of the 204 bp *Pvu*II – *Bgl*II fragment from which the *ohm1* mutation can be rescued. As a control, we also determined the DNA sequence of the analogous DNA fragment from a wild-type λ control. The sequence we determined for the wild-type fragment was identical to the published λ sequence (Sanger et al. 1982). In the mutant fragment, we found a single change at base pair 308; a C:G bp was changed to a T:A bp. This transition alters the 40th codon of *Nul*, resulting in the replacement of a leucine residue with a phenylalanine at this position of gp*Nul*.

Discussion

The experiments reported in this paper address, in part, the question of the role of host factors in the action of λ terminase. Previous studies with phage 21 demonstrated

an absolute requirement for IHF in the action of the terminase of that phage and, by analogy, suggested that IHF might play a role in the action of λ terminase (Feiss et al. 1985a). In vitro studies demonstrated that terminase action requires a host component that can be contributed either by IHF or another DNA-binding protein, THF (Gold and Parris 1986). Because the gene for THF has not been identified, the physiological role for this protein remains obscure.

IHF was first identified in another set of reactions which also involve both a specific site on λ DNA and λ-encoded proteins; the site-specific recombination reactions that integrate and excise λ from the *E. coli* chromosome (Mizuuchi and Nash 1976). Indeed, mutations in the genes encoding the two IHF subunits were identified because of the failure of the mutant bacteria to support λ site-specific recombination (Miller and Friedman 1977; Kikuchi et al. 1985). Subsequent studies have shown that IHF influences gene expression (Miller 1981; Friedman et al. 1984a; Friden et al. 1984; Dorman and Higgins 1987) as well as playing a role in other site-specific recombination reactions (Prentki et al. 1987; Eisenstein et al. 1987; Drlica and Rouviere-Yaniv 1987).

The marker rescue experiments locate *ohm1* in the *Nul* gene and demonstrate that all genetic information necessary and sufficient for the Ohm phenotype is located in a 204 bp fragment that contains the amino portion of *Nul*. Sequence analysis revealed that the only change in the fragment isolated from *λohm1* is a single transition in *Nul* that results in an amino acid substitution at codon 40. It is interesting that Feiss et al. (1987), using a different selection procedure, isolated the identical mutation, which they call *ms1*.

Complementation studies demonstrate that the *ohm1* mutation is dominant, arguing that there is a gene product involved. Taken together with the mapping and sequencing studies, these results lead us to conclude that the *ohm1* allele encodes a mutant *Nul* subunit and, in turn, leads to the synthesis of a mutant terminase with novel properties.

Our studies using packaging of dilysogens (the Ter test) as the indicator demonstrate that wild-type terminase is more efficient (by a factor of 3) when the bacterium expresses IHF. This difference in packaging is enough to account for the reduced burst of λ in IHF-defective bacterium (*himA* mutants). The Ter tests also show that the *ohm1* terminase packages wild-type λ DNA efficiently under *himA*$^+$ (IHF present) or *himA*$^-$ (IHF absent) conditions. These results suggest that *ohm1* renders terminase IHF-independent.

The experiments with *cos154* offer further support to the argument for the IHF-independence of the *ohm1* terminase. When λ DNA with the *cos154* mutation is the substrate for the terminase reaction, wild-type terminase is significantly more dependent on IHF. However, the *ohm1* terminase appears to be IHF-independent in its action at the mutant *cos*.

In addition to IHF, our experiments also implicate DNA gyrase in terminase activity. There are many reasons why the *gyrB-him230*(Ts) mutation might reduce terminase activity. First, the pleiotropic nature of this mutation could result in the reduced synthesis of one or more λ or host proteins involved in DNA packaging. This idea is based on the observation that DNA gyrase mutations alter the specificity and strength of λ (Botchan et al. 1973) and *E. coli* (reviewed by Smith 1981) promoters. Second, the mutant DNA gyrase (or β subunit) could bind to *cos* in a nonproductive manner and negatively complement terminase; mutations of this type, involving a phage-encoded topoisomerase, have been described for T4 packaging (Zachary and Black 1986). Third, terminase could require a supercoiled substrate for optimal activity, as is the case for several site-specific recombination systems (Reed 1981; Isberg and Syvanen 1982). Two lines of evidence consistent with this idea are: (1) λ circular DNA is relaxed in *himAΔ82/gyrB-him230*(Ts) bacteria at 42° C (Friedman et al. 1984a), and (2) in vitro, supercoiled DNA containing *cosB* binds terminase more efficiently than the analogous linear DNA (Feiss et al. 1983).

The fact that the *ohm1* mutation is sufficient to permit the phage to overcome the block imposed by the double *him* mutant argues that the inhibition of phage growth results from a failure in terminase activity. Interestingly, the *gyrB-him230*(Ts) mutant was isolated, as were the *himA* and *hip/himD* mutants, by a selection for *E. coli* which failed to support λ site-specific recombination. Thus, two host-encoded proteins that contribute to λ site-specific recombination are also involved in terminase reactions.

Remarkably, we can extend the analogy between *cos* cleavage and λ site-specific recombination to include the *ohm1* mutation. Mutations in the *int* gene, *int*-h3 (Miller et al. 1980) and *xin* (C. Gritzmacher, L. Enquist and R. Weisberg, personal communication), result in the synthesis of an integrase capable of functioning independently of IHF. As in the case of *ohm1* and *ms1*, *int*-h3 and *xin* were isolated using different selection procedures, yet have precisely the same base change (S. Bear et al. 1987).

Other phage proteins for which IHF-independent mutants have been isolated include the terminase of phage 21 (Feiss et al. 1985a) and the "transposase/replicase" of phage Mu (Yoshida et al. 1982). From a deterministic point of view it seems reasonable to assume that there are selective pressures operating so that these proteins evolved with requirements for participation of IHF in their action. We suggest that IHF may play a regulatory role in λ packaging, as it does in λ integration (Miller 1981).

The studies with the *cos154* prophage also address the question of the processivity of the terminase reaction. Previous studies have demonstrated that in the terminase-catalyzed packaging of concatameric λ DNA, a *cosB* site is only required for the initial recognition of terminase (Feiss and Widner 1982; Feiss et al. 1985b). Our studies demonstrate that the *cos154* mutation only influences the terminase reaction when it is in the initial *cos* recognition sequence (see Fig. 2); once terminase binds, subsequent *cos* sites can be cleaved even if they contain the *cos154* mutation. This observation argues that the *cos154* mutation influences the initial recognition of terminase. Since the effect of *cos154* is only observed in the absence of IHF, it has been argued that the *cos154* mutation, which changes 1 bp in a region of 16 bp perfect hyphenated dyad symmetry, defines a recognition site, either for a host factor (such as THF) that can substitute for IHF, or for terminase. In the latter case, IHF would facilitate binding of terminase to a *cosB* with an altered recognition sequence.

In any event, since the *ohm1* terminase is active on DNA containing the *cos154* mutation in the absence of IHF, the amino acid substitution in gp*Nul* corresponding to the *ohm1* mutation might result in a terminase that binds *cosB* more effectively. The reader is referred to the companion paper by Feiss et al. (1988) for a discussion of the possible mecha-

nistic changes imposed on terminase by the *ohm1/ms1* mutation.

In conclusion, the isolation of the *ohm1* mutation demonstrates that in vivo the terminase reaction requires both IHF as well as DNA gyrase activity for maximum efficiency.

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