Cell, Vol. 20, 711-719, July 1980, Copyright © 1980 by MIT

An E. coli Gene Product Required for λ Site-Specific Recombination

Harvey I. Miller* and David I. Friedman
Department of Microbiology and Immunology
University of Michigan
Ann Arbor, Michigan 48109

Summary

We report characteristics of *himA* mutations of E. coli, selected for their inability to support the site-specific recombination reaction involved in the formation of lysogens by bacteriophage λ. The *himA* allele lies at minute 38 on the chromosome. Three noncomplementing and closely linked mutations define the *himA* locus; one is a nonsense mutation which shows that the gene product is a protein. *HimA* mutations reduce both λ integrative and excisive site-specific recombination. Since dominance tests demonstrate that *himA* mutations are recessive, it is probable that the *himA* protein is either a necessary component for site-specific recombination or, alternatively, regulates the expression of such a function. *HimA* mutations exhibit pleiotropic effects. They reduce integration of phages that have different attachment specificities from λ and inhibit the growth of phage mu. In addition, *himA* mutations reduce precise excision of integrated phage mu as well as Tn elements. This pleiotropy suggests that the role of *himA* protein is nonspecific. Since all of the processes affected by *himA* mutations ultimately rely on protein-DNA interactions, we suggest that *himA* protein may act in an auxillary manner to facilitate these interactions.

Introduction

An obligatory step in the formation of stable lysogens by coliphage λ is the integration of the phage genome into the E. coli chromosome (Campbell, 1962; for recent reviews, see Nash, 1977; Weisberg, Gottesman and Gottesman, 1977). The integrated phage DNA directs the synthesis of a repressor protein which binds to specific sites on the prophage DNA and blocks the expression of most other phage products (Ptashne et al., 1976). When repression is removed, phage functions can be expressed and the DNA is efficiently excised from the bacterial chromosome, ultimately resulting in production of mature phage particles.

Both genetic and in vitro studies have identified several proteins and DNA sites involved in the integration and excision of the λ genome. Integration and excision normally occur at a series of unique nucleotide sequences (*att* or attachment sites) located on the phage (*att*P) and bacterial chromosome (*att*B), or flanking the integrated prophage (*att*L and *att*R). One phage-encoded protein, integrase (Int), is required for integration (Zissler, 1967; Gottesman and Yarmolinsky, 1968; Echols, Gingery and Moore, 1968), and another excisionase (Xis) is additionally required for excision (Guarneros and Echols, 1970). Other temperate coliphages capable of lysogeny by integration exhibit different attachment site and Int specificities (Gottesman and Yarmolinsky, 1968; Bertani, 1970).

The integration and excision of λ occurs normally in the absence of host-generalized or homology-dependent recombination functions. Indeed, the extent of homology between the four *att* sites is limited to a 15 nucleotide core sequence that is common to all four *att* sites (Shulman and Gottesman, 1973; Landy and Ross, 1977).

Recombination in the absence of host-generalized recombination functions has been observed in E. coli with several genetic elements. This recombination generally appears to occur at unique nucleotide sequences and is thus referred to as site-specific recombination. In contrast to some temperate bacteriophages such as P2, ϕ80 and λ that recombine with the E. coli chromosome at specific phage and bacterial sites, recombination between the E. coli chromosome and other genetic elements such as the temperate bacteriophage mu (Taylor, 1963; Howe and Bade, 1975; Bukhari, 1976), transposable antibiotic resistance factors (Tn elements) (Berg et al., 1975; Kleckner et al., 1975; Berg, 1976), and *IS* (insertion sequences) (Starlinger and Saedler, 1972) appears to occur at specific nucleotide sequences located only on the element and more randomly with respect to the bacterial chromosome (reviewed by Starlinger and Saedler, 1976; Kleckner, 1977).

The prevalence of site-specific recombination processes active in E. coli led us to consider the possible existence of bacterial functions participating in these events. That host-encoded proteins participate in site-specific recombination in vitro was shown by Mizuuchi and Nash (1976) and confirmed by the isolation of bacterial mutants unable to support phage integration, *him*A, *him*B, *hip* and *him*C (Miller and Friedman, 1977; Williams, Wulff and Nash, 1977; Miller et al., 1979). P1 transduction studies have located the *him*A mutations in the *aro*D-*his* region at minute 38 (Miller et al., 1979). We now analyze the complementation patterns of *him*A mutations as well as the effects of *himA* mutations on recombination processes related to λ site-specific recombination.

Results

Complementation Studies of *him*A Mutations

Merodiploid *him*A$^-$ (*him*A$^+$) derivatives were constructed by introducing the *him*A$^+$ allele into *him*A$^-$ strains using a specialized transducing phage which

* Present address: Department of Molecular Biology, University of California, Berkeley, California 94720.

has been shown to carry the *phe*S gene as well as other genes in this region (Hennecke, Springer and Back, 1977a; Hennecke et al., 1977b; Springer, Graffe and Hennecke, 1977). In each case, the diploid strains were Him$^+$, indicating that Him$^+$ is dominant to Him$^-$ and confirming that the transducing phage carries the *him* gene. Using these lysogens, variants of the transducing phage carrying the various *him*A mutations were isolated (see Figure 1). To simplify our discussion, this phage will be referred to as λ*him*A.

Complementation between the various *him*A mutations (*him*A79, *him*A42 and *hid*) was studied using *him*A$^-$ derivatives of the λ*him*A phage. Mutant *him*A$^-$ strains were lysogenized with each of the *him*A$^-$ phage. Regardless of the combination of mutations, the lysogens were phenotypically Him$^-$; that is, there was no complementation. This finding is consistent with the placement of the *him*A mutations in the same cistron. This explanation is favored, since three obvious alternative explanations can be ruled out. First,

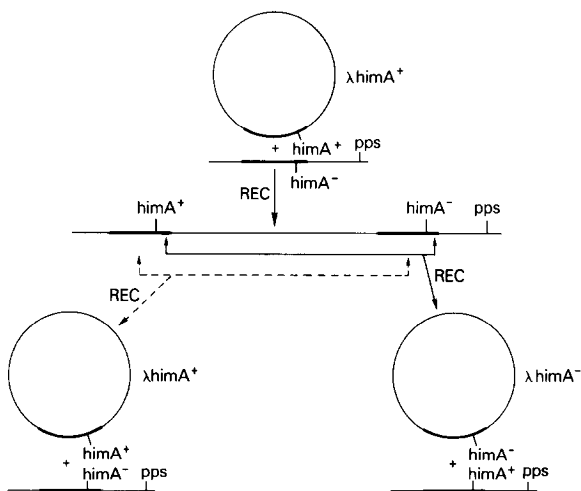

Figure 1. Integration and Curing of λ*himA*

The λ*himA* phage is deleted for the majority of the *int* gene and therefore cannot integrate via the normal integration pathway (Kourilsky et al., 1978). Since the phage carries bacterial DNA, lysogens can be formed by homologous (Rec-promoted) recombination into the *himA* region, the recombinational event occurring anywhere within the region of homology. The resultant lysogens carry a duplication of the *himA* region flanking the integrated prophage. A recombinational event anywhere within the duplicated segment of bacterial DNA can lead to a bacterium cured of the prophage. Thus although originally the bacterium carried the mutant allele (*himA*$^-$), cured bacteria can be recovered carrying the wild-type (*himA*$^+$) allele, providing the recombination occurred to the right of the mutation as shown. Cured bacteria are selected as survivors of growth at 40°C. Since induction of the λ*himA* prophage (which expresses the thermoinducible cI857 repressor) is lethal to the cell, only bacteria that have lost the prophage prior to the temperature shift survive at high temperature.

Using thermoinduction, phage carrying the mutant allele can be isolated from lysates of lysogenic strains. Although these lysates contain both *himA*$^+$ and *himA*$^-$ phage, the latter can be recovered by lysogenizing the homologous *himA*$^-$ host with the mixed lysate and screening for a phenotypically *himA*$^-$ lysogen. These lysogens must carry a duplication of the *himA*$^-$ allele. Induction of these lysogens yields a homogeneous population of phage carrying the *himA*$^-$ allele.

*him*A$^-$ mutations do not negatively complement, since *him*$^+$ hosts carrying λ*him*A$^-$ prophages are phenotypically Him$^+$. Second, the transducing phages do not appear to lack a promoter for the *him*A gene. The absence of this promoter would be detected in studies with λ*him*A derivatives; the Him$^-$ phenotype would be observed either in most λ*him*A$^+$ lysogens of *him*A$^-$ strains or in some λ*him*A$^-$ lysogens of *him*$^+$ hosts. In one of these two types of lysogens, the wild-type allele would be separated from its promoter. Since Him$^+$ lysogens are always found, irrespective of the initial location of the *him*A$^+$ allele, both *him*A genes must be associated with active promoters. Third, since the phage were generated by homologous recombination, it would seem improbable that this should cause a loss of genetic material. This was directly proven for phage carrying the suppressible mutation *him*A*am*79. Under nonsuppressing conditions, *him*A42 hosts lysogenized with λ*him*A*am*79 are phenotypically Him$^-$. If the strains are further lysogenized with ϕ80*su*III$^+$, however, the lysogens now become Him$^+$. At least in the case of the λ*him*A*am*79 phage, therefore, the transducing phage can be shown to have the *him*A gene intact.

We conclude that *him*A*am*79, *him*A42 and *hid* all map in the same cistron, because of the absence of complementation, the close map positions, and the limited coding capacity of the bacterial DNA carried by the λ*him*A phage (Springer et al., 1977).

Him Effects on Site-Specific Recombination

Integrative recombination was studied in hosts carrying either the *him*A42 or *him*A*am*79 mutations in three ways: stable lysogen formation; intermolecular Int-promoted recombination; and conversion of λ*att*B-*att*P. The results of these experiments are listed in Table 1. Although the procedures used to measure these three processes are included in Table 1, we note the following about the third: λ*att*B-*att*P is one of the class of phages known as λ*att*^{2} that carry bacterial genes flanked by substrates for site-specific recombination (Shulman and Gottesman, 1971; Nash, 1974). In the presence of the requisite recombination functions, the genomes of these phages lose their bacterial DNA, which results in the production of phage identifiably different from the parental phage. The conversion frequency of phage with large genomes to phage with small genomes serves as a quantitative measure of site-specific recombination. Conversion of λ*att*B-*att*P requires Int, while conversion of λ*att*L-*att*R requires Xis and Int.

As shown in Table 1, measurements of integrative recombination in a strain carrying the *him*A42 mutation reveal a virtually total defect in this type of recombination as measured either by frequency of lysogeny, intermolecular *att*B × *att*P recombination (in phage-phage crosses), or intramolecular recombination (in λ*att*B-*att*P), even when Int is provided by a λ*int*-c

Table 1. Integrative Recombination in Him Mutants

		% Integrative Recombination				
		Intermolecular				
		Lysogeny			Intramolecular *attB* × *attP*[d] Int Supplied by	
Bacteria	Allele	Nonimmune[a]	Immune[b]	*attB* × *attP*[c]	*int*+	*int*-c
K37	*himA*+	>90	>90	8	34	58
K648	*himAam79*	0.1	nt	0.1	0.2	4
K634	*himA42*	0.01	1.0	0.1	0.1	0.1

Bacteria were grown to 2 × 10^8/ml in LBMM, pelleted, and resuspended at the same concentration in 10 mM $MgSO_4$. For the standard EDTA treatment used to detect recombinant phage with smaller genomes (Parkinson and Huskey, 1971), lysates are diluted 100 fold into TE buffer and incubated at 41°C for 15 min. The $MgSO_4$ concentration is adjusted to 20 mM prior to titering with 1 M $MgSO_4$.

[a] The frequency of lysogeny was determined using λcI857 as the test phage. (See Experimental Procedures).

[b] Both *him*+ and *himA42* lysogens of λcI857*Pam3att80* were grown at 30°C and then infected with λc+*int*-c226 at an moi of 10. The infected cells were diluted 10^{-3} and grown overnight at 30°C. Appropriate dilutions were plated onto TB plates, and duplicate plates were incubated either at 30°C to determine the total viable cells or at 41°C to determine the number of cells that carried λc+*int*-c. Since the original prophage carries the cI857 mutation, the repressor expressed is thermolabile and the lysogens are induced at 40°C. If the lysogen is subsequently lysogenized by the λc+*int*-c phage, however, the lysogen will no longer be thermoinducible and thus will survive at 40°C. Survival of uninfected lysogens at 40°C is 1%.

[c] Intermolecular integrative recombination was measured using λcI857 (*attP*) and λcI857*gal49bio256*(*attB*). One of the recombinants resulting from a cross of these two phages, λcI857*gal49* is dinstinguishable from both parental phages because it has a smaller genome that renders it insensitive to EDTA treatment, and because it carries the λ*red* region and is thus able to plate on *recA*− hosts (the Fec+ phenotype—Zissler, Signer and Schaefer, 1971).

Bacteria were infected with both phages at an moi of 5 of each phage. After a 10 min adsorption, the infected cells were diluted into prewarmed LBMM and incubated 90 min at 37°C. Chloroform was added and total Fec+ phage were assayed on a *recA*− lawn (K100), and recombinant phage were assayed on the same lawn following EDTA treatment. Recombination is expressed as EDTA resistant-Fec+ phage × 100 per total Fec+ phage.

[d] Intramolecular integrative recombination was measured using λ*attBattP*. Bacteria were infected with this phage at an moi of 1. Int was supplied in trans by co-infecting at an moi of 5 with either λN−*int*-c or λcI857 (*int*+). Total λ*imm21* phage were assayed on a lawn of a λ lysogen (K124), and recombinant phage were assayed on the same lawn following EDTA treatment. Recombination is expressed as EDTA resistant phage × 100 per total phage.

Results shown are the average of at least two determinations. In each case, adsorption was greater than 90%. Burst sizes were equivalent for all experiments. Phage stocks of λ*attB-attP* and λcI857 initially contained 0.1% EDTA-resistant phage.

phage, a mutant shown to produce high levels of integrase (Shimada and Campbell, 1974; Katzir et al., 1976). In the case of lysogeny, the fact that similar results are observed in the presence of constant immunity (Table 1, column 4) suggests that phage growth and establishment of repression per se do not influence to any great extent the outcome of measurements of integration. Strains carrying the *himAam79* mutation are also extremely defective for all forms of integrative recombination, but not as defective as *himA42*-containing strains. The restriction by the *himAam79* mutation on integrative recombination assayed by λ*attB-attP* is partially overcome if *int*-c is used to supply Int.

Excisive recombination, as measured by conversion of λ*attL-attR* (Shulman and Gottesman, 1971) is also inhibited in *himA*− hosts. As shown in Table 2, the *himA* mutations reduce this conversion reaction by a factor of 20 fold. The effects of *himA* mutations on excision were also measured by determining the burst of phage following thermoinduction of λcI857 monolysogens, an assay ultimately dependent on the efficient excision of the prophage. By this test, excision is reduced by 5 orders of magnitude (Table 2), equivalent to the reduction caused by *xis*− mutations (Guarneros and Echols, 1970). Surprisingly, excisive recombination measured by curing of a λcI857 prophage by transient thermoinduction (Weisberg and Gallant, 1967) shows only a 2 fold reduction in *himA*− hosts. This suggests that prolonged exposure to Int and Xis, an inherent feature of the transient induction curing method, overcomes the *himA* requirement for excisive recombination. On the other hand, prolonged exposure to Int does not bypass the requirement for the *himA* product in integrative recombination, since λ*int*-c phage still fail to lysogenize *himA*− hosts.

When the normal bacterial attachment site, *attB*, is deleted, λ can integrate at a limited number of secondary sites (Shimada, Weisberg and Gottesman, 1972, 1973). The affinity of λ for these secondary sites is 200 fold lower than its affinity for the normal *attB* site. The excision of a λ prophage from a secondary attachment site can be qualitatively assayed using the red plaque test (Enquist and Weisberg, 1976; see also Experimental Procedures).

A *himA42* derivative of a red plaque assay strain was constructed (K713). Since *int*+*xis*+ phages form colorless plaques on this strain, it is concluded that

Table 2. Excisive Recombination in Him Mutants

		% Excisive Recombination		
Bacteria	Allele	Curing[a]	Intramolecular[b] *attL* × *attR*	Average Phage Burst[c]
K37	*him*A$^+$	85	68	100
K648	*him*A*am*79	40	3	nt
K634	*him*A42	58	2	0.001

[a] Prophage curing was determined by measuring the percent loss of a λcI857 *Pam*3 prophage following transient heat pulse. Since the cI857 mutation specifies a reversibly thermosensitive repressor, transient heat pulses result only in expression of some early phage genes, including *int* and *xis*, before repression is reestablished (Weisberg and Gallant, 1967). Relatively efficient phage excision can therefore, occur even though there is insufficient expression of lethal phage functions to cause cell death.

Derivatives of a K37 λcI857 *Pam*3 lysogen that are *him*$^+$ (K661), *him*A42, (K663) and *him*A79 (K664) were grown in LB at 32°C and diluted into 41°C broth for exactly 6 min. Samples were then diluted into 32°C broth in which they were grown overnight with aeration. Dilutions were spread on TB plates and incubated overnight at 32°C. Single colonies were tested with λCI60 to detect nonlysogens. Curing is expressed as cured cells × 100/total.

[b] Intramolecular excisive recombination was measured as described in the legend to Table 1, except that λ*attL-attR* was used at an moi of 1. Int and Xis were supplied in trans by λcI857 (*int*$^+$*xis*$^+$) at an moi of 5.

[c] Lysogens of the indicated strains carrying a λcI857 prophage were grown in LB at 32°C to a density of 2 × 10^8 per ml. The cells were diluted 10^{-3} in LB that had been prewarmed at 41°C. After a 90 min incubation at the high temperature, chloroform was added and the burst was assayed. Burst is calculated as phage per induced cell.

Bursts of λcI857 following infection of either *him*$^+$ or *him*A$^-$ nonlysogens at 41°C did not vary by more than a factor of 2 (data not shown). (nt) not tested.

excision of λ from this secondary *att* site is defective in the *him*A42 mutant strains. Moreover, as discussed in the accompanying paper (Miller, Mozola and Friedman, 1980), a λ mutant that shows normal levels of integration in *him*A mutants forms a red plaque on K713.

Relation of Him and Rec

Measurement of recombination in Him$^-$ hosts shows that Him functions are not involved in generalized recombination. P1 transduction or Hfr transfer resulted in the stable transfer of markers into *him*A$^-$ strains at the same frequency as the transfer into isogenic *him*A$^+$ strains. A more sensitive assay was provided by measuring recombination between two λ phages (see legend to Table 3). As shown in Table 3, no significant difference was observed in the frequencies of generalized recombination between phage mutants.

Growth and Integration of Bacteriophage Mu in Him Mutants

Bacteriophage Mu, which integrates at random sites in the E. coli chromosome when establishing lysogens, appears to require integration also for lytic growth

Table 3. Generalized Recombination in Him Mutants

		Titer on:		
Bacterium	Allele	K60	K37	% Recombination
K37	*him*A$^+$	4.7 × 10^9	3.6 × 10^8	7.6
K634	*him*A42	3.7 × 10^9	1.4 × 10^8	3.8

Bacteria grown to 2 × 10^8/ml in LBMM were pelleted and resuspended in 10 mM $MgSO_4$. The cells were infected with λcI_{857} *Nam*7*am*53 *red*3 and λcI_{857} *cam red*3 *Sam*7, each at an moi of 5. After a 10 min absorption period, the cells were diluted 10^3 fold into prewarmed LBMM and grown for 90 min, at which time $CHCl_3$ was added. The phage burst was titered on K60(*su*III$^+$) and K37(*su*^{0}). Both parental phage plate on K37 with an efficiency of 10^{-5}. The *red*$^-$ mutation eliminates the phage-generalized recombination system (Signer et al., 1968). Because the amber mutations are both situated on the same side of *att*, there is no effect of site-specific recombination (see Figure 1).

(Taylor, 1963; Howe and Bade, 1975; reviewed by Bukhari, 1976). It was therefore, not surprising to find that lytic growth of Mu was severely reduced in *him*A$^-$ mutants, observed as a failure of plaque formation. Since adsorption of Mu to the *him*A$^-$ strains is normal, the failure to grow must reside in a post-adsorption step. Phage burst was used as a more quantitative measure of this inhibition. Mu*cts*-62 (a variant that expresses a thermolabile repressor) growth was initiated either by infection of cells (at low moi) or by induction from the prophage state. Mu*cts* lysogens of *him*A$^-$ bacteria were constructed by introducing the *him*A mutation into a Mu*cts* lysogen by co-transduction with *pps*. These lysogens were still Mu-immune, as evidenced by their inability to plate Mu*nu*A, a Mu variant selected for its ability to plate on *him*A (see below). Table 4 shows the results of single-cycle growth experiments. Regardless of how Mu growth was initiated, the burst in the *him*A42 strain was 10^{-2}–10^{-3} particles per induced or infected cell. In analogous experiments, the burst in a *him*$^+$ strain was approximately 1–2 × 10^2 particles per induced or infected bacterium.

Further emphasizing the restrictive effect of the *him*A42 mutation on Mu growth is the observation that *him*A42 strains survive both thermoinduction of *him*A42 (Mu*cts*-62) and infection by Mu at inducing temperatures, whereas isogenic *him*A$^+$ strains treated in the same manner are killed, survivors being found at a frequency of ~10^{-5}. However, the colony morphology of induced *him*A (Mu*cts*) lysogens is noticeably different from that of nonlysogens or noninduced lysogens. The induced *him*A42 lysogens carrying Mu*cts*-62 in the *lac* operon retain the prophage in its initial position. The lysogens are *lac*$^-$, and the fact that they remain so after induction implies that the Mu position has not changed, an implication confirmed by the following experiment. Several *him*A (Mu*cts*) lysogens were grown at an inducing temperature for three successive single colony isolations, and then at a

Table 4. Growth and Integration of Mu in Him Mutants

Bacterium	Allele	% Lysogeny[a]	Average Burst		Complementation	
			Infection[b]	Induction[c]	−Mu*nu* A[d] Average Burst of Mu*cts*-62	+Mu*nu*A[e] Average Burst of Mu*cts*-62
K37	*him*A$^+$	17	200	50	125	
K634	*him*A42	12	0.05	0.002	0.57	48

Cells were grown in LB + 10 mM $MgSO_4$ + 5 mM $CaCl_2$ to a concentration of 2×10^8/ml at 32°C for induction experiments or at 37°C for the infection experiments.

[a] To measure the frequency of lysogeny, cells were infected at 32°C with Mu-*cts*-62 at an moi of 5. After a 15 min adsorption, dilutions of the infected cells were spread on TB plates and incubated overnight at 32°C. Colonies were scored for Mu lysogeny by determining whether they were "immune" to Mu infection by cross-streaking the bacteria against Muc25. This test could be used in the case of *him*A$^-$ mutants because there was an observable lysis of the mutant bacteria by Muc25 (>10^{10} pfu/ml) even though this phage does not form a plaque on a lawn of *him*A$^-$ bacteria. As a further verification for lysogeny, each colony was purified and tested for spontaneous release of phage.

[b] Cells were infected with Mu*cts*-62 at an moi of 0.1 at 41°C and adsorption was allowed for 15 min. The infected cells were diluted 10^3 fold into prewarmed LB at 41°C. After 90 min, $CHCl_3$ was added and total phage was titered on K37 on TCMG plates. Average burst is calculated as total phage per infected cell.

[c] Mu*cts*62 lysogens of K37 (K686) and K634 (K655) were induced by diluting the culture into prewarmed LB at 41°C. After 90 min, $CHCl_3$ was added and total phage was titered on K37. Burst is calculated as total phage per induced cell.

[d] Bacteria were infected with Mu*cts*-62 at an moi of 5. The burst was determined as described in footnote c.

[e] Bacteria were infected with at an moi of 5 each with Mu*cts*-62 and Mu*nu*A. Infection procedures were the same as outlined above. Plaques were tested for growth on a *him*A$^-$ strain (K634) to distinguish the two phage types. The burst size was 100 when Mu*nu*A was used and consisted of ~50% of each phage type.

noninducing temperature (32°C) for one isolation. Then, using P1 grown on a *lac*$^+$ nonlysogen, the lysogens were transduced to *lac*$^+$. In every case the *lac*$^+$ transductants had lost the Mu prophage.

The frequency of lysogeny by Mu*cts*-62 in Him$^+$ and Him$^-$ hosts was determined as described in Table 4. Contrary to our expectations, the recovery of immune lysogens among the survivors of infected cells was not significantly different in the *him*A$^-$ strain from the wild-type isogenic host. The ability to recover immune lysogens of *him*A$^-$ bacteria indicates that Mu can express functions necessary for establishment and maintenance of lysogeny as well as for integration.

The *him*A$^-$ lysogens of Mu*cts*-62 (or of Muc$^+$) spontaneously produce low levels of phage. The amount of phage produced is independent of the growth temperature and approximates the low level of phage spontaneously produced from wild-type uninduced lysogens (Howe and Bade, 1975). In addition, the temperature independence indicates that phage production in *him*A$^-$ strains is not due to repressor inactivation.

Excision of Mu from a Bacterial Gene

Biological studies on Mu excision cannot be performed using wild-type Mu because the excision event itself is probably lethal to the bacterial host. Bukhari (1975) has developed a method for studying Mu excision using the defective Mu variant, MuX, which surmounts this difficulty. Excision of MuX is not a lethal event and occurs in either a precise or an imprecise manner. When the MuX prophage is integrated in the *lac*Z gene, only precise excision results in the restoration of an intact *lac*Z gene, forming lac$^+$ revertants. Imprecise excision may relieve the Mu-induced polarity on the expression of the promoter-distal *lac*Y gene, however, forming *lac*$^-$ *mel*$^+$ revertants. Using this system, the frequency of both precise and imprecise excision of MuX was compared in wild-type (K650) and *him*A42 derivatives (K783) of a lacZ::Mux lysogen. The *lac* operon was present on an F'*pro lac* episome. The frequency of both types of revertants is compared in Table 5. *Lac*$^+$ revertants are at least 10^3 fold less frequent and *mel*$^+$ revertants at least 10^4 fold less frequent in K783 than in K650. Depending on how closely MuX excision mimics normal Mu excision, this experiment suggests that *him*A mutations interfere with Mu excision.

Reversion of Tn Insertion Mutations

Transposable antibiotic resistance elements (Tn elements) induce insertion mutations in bacterial operons in a manner similar to bacteriophage Mu. Since reversion of MuX-induced mutations was severely reduced in *him*A$^-$ bacteria, we examined the reversion of a Tn10 (tetracycline resistance—Kleckner et al., 1975) induced insertion mutation in the E. coli *pro* operon. As shown in Table 5, reversion of *pro*::Tn10 mutations is reduced 10 fold in the *him*A42 host (K5078) as compared with the wild-type isogenic host (K5076) when the *pro* operon is located on an F*pro-lac* episome. When the same insertion is chromosomally located, we cannot detect a significat difference in reversion frequencies, perhaps because of the large variation in the frequencies observed at these extremely low reversion rates. Table 5 also indicates that there is a more than 100 fold increase in the

Table 5. Effect of the *him*A42 Mutation on Excision of Mu and Tn10

	Genotype			
Strain	Chromosome	Episome	Reversion	Polarity Relief
K650 *him*A$^+$	Δ (*pro-lac*)	F′ *pro lacZ*::muX5004	$^a2.0 \times 10^{-7}$	$^b2.0 \times 10^{-6}$
K783 *him*A42	Δ (*pro-lac*)	F′ *pro lacZ*::muX5004	$^a<2.0 \times 10^{-10}$	$^b<2.5 \times 10^{-10}$
K5076 *him*A$^+$	Δ (*pro-lac*)	F′ *pro*::*Tn10*-580 *lac*	$^c5.2 \pm 0.6 \times 10^{-6}$	nt
K5078 *him*A42	Δ (*pro-lac*)	F′ *pro*::*Tn*10-580 *lac*	$^c0.6 \pm 0.1 \times 10^{-6}$	nt
K5096 *him*A$^+$	*pro*::*Tn*10-580		$^c2.5 \pm 0.8 \times 10^{-8}$	nt
K5097 *him*A42	*pro*::*Tn*10-580		$^c0.7 \pm 0.3 \times 10^{-8}$	nt

Bacteria were grown to saturation in LB at 37°C, washed twice with 10 mM $MgSo_4$, and incubated at 32°C for 30 min. Dilutions were spread on the following sets of plates: (a) Minimal glucose + trp and minimal lactose + trp at 37°C. (b) Minimal glucose + trp and minimal melibiose + trp at 43°C. (c) Minimal lactose + pro and minimal lactose at 37°C. Frequencies are expressed as titer on second set of plates per titer on first set of plates. Numbers are the average of at least three determinations. (nt) not tested.

reversion frequency for the identical *pro*::Tn10 insertion when it is located in the episome rather than the chromosome. We have determined that the episome effect is a *cis* effect. That is, the reversion frequency of a chromosomal Tn10 insertion (at a different location) cannot be increased by the presence of an F′ episome carrying the same Tn element (data not shown). We have noticed a similar phenomenon with the Tn5 (kanamycin resistance—Berg, 1976) elements (data not shown). Although reversion of Tn insertion mutations is reduced by *him*A mutations, this effect is far less striking than that seen with MuX (see Discussion).

Mu Variants Capable of Growth on Him Mutants

Mutants of Mu*cts*-62, called Mu*nu*A, that grow on Him$^-$ strains have been isolated from stocks of Mu*cts*-62 grown on the mutator strains, *Mut*D5, (Fowler, Degnen and Cox, 1974). Such mutants have not been obtained without mutagenesis, and even with mutagenesis are found only at a frequency of $\sim 10^{-9}$. No other phenotypic alterations are discernable, however, lytic growth, lysogeny, and expression of the thermoinducible repressor carried by the parental phage are all normal. Experiments with *nu*A variants and wild-type phage demonstrate that Mu*nu*A-1 is capable of helping wild-type Mu for growth in *him*A42 strains. Co-infection of a *him*A42 strain with equal numbers of Mu*cts*-62 and Mu*nu*A-1 results in a normal phage burst consisting of equal numbers of both input phage (Table 4). This suggests that *nu*A variants are able to supply some function(s) in trans that permits growth of wild-type Mu in the *him*A$^-$ mutant.

The Mu*nu*A phages are capable of growing both on strains carrying the *him*A42 mutation and on those carrying the *him*A*am*79 mutation. Mapping studies indicate that the *nu*A mutations are located near or in the MuA gene (R. Yoshida et al., manuscript in preparation).

Effects on Repressor Synthesis

As previously mentioned, λ forms somewhat clear plaques on *him*A$^-$ strains, indicating some defect in either of the two modes of repressor synthesis, establishment or maintenance. λ*imm*21 and λ*imm*434, as well as λcI*ind*$^-$ (noninducible), display this phenotype.

Since λ lysogens of *him*A$^-$ hosts show normal stability, the clear plaque phenotype is not likely to result from a defect in the maintenance of repression. This conclusion is confirmed by direct measurements of gene expression from the promoter-controlling maintenance expression, P_{RM}. These studies show identical levels of gene expression from P_{RM} in both the *him*A$^-$ and *him*A$^+$ hosts (H. Miller, unpublished observations). This implies that the clear-plaque phenotype might be a consequence of an effect of *him*A$^-$ on the expression from the promoter-controlling establishment of repression, P_{RE}. Consistent with such an interpretation is the observation that at 30°C λcI857*cro*$^-$, which fails to plate on *him*A$^+$ hosts, plates on *him*A$^-$ hosts. The failure of the *cro* mutant to plate on wild-type strains at 30°C is due to an overproduction of repressor following infection (Eisen et al., 1970; Reichardt and Kaiser, 1971). This suggests that the ability of λ*cro*$^-$ to plate on *him*A$^-$ hosts at 30°C reflects a defect in the establishment mode of repressor synthesis.

These experiments indicate that *him*A product may influence the level of activity of P_{RE}, the normal promoter for establishment of repression.

Discussion

The *himA* Gene

The experiments reported in this work identify an E. coli gene, *him*A, whose product is required for several types of site-specific recombination. Thus *him*A mutants are representative of a class of recombination-deficient mutants that are functionally and genetically distinguishable from mutants defective in generalized recombination.

The *him*A gene probably constitutes a single cistron and encodes for a protein. These conclusions are based on the observations that three independently isolated mutations fail to complement, and that one of these is a nonsense mutation.

Site-Specific Recombination

We have demonstrated a requirement for the *him*A gene product in all tested forms of λ site-specific recombination. Our results indicate that the failure of *him*A mutants to support site-specific recombination of λ is due to the absence of an active host protein that participates in the recombination reaction. The following observations argue against the possibility that *him*A mutations affect a process that secondarily influences in vivo measurements of site-specific recombination.

First, we have assayed λ integrative recombination by several methods in addition to lysogeny. One method (λ*att*^{2}) requires only lytic growth of the test phage, and another (integration of λ*int*-c under immune conditions) requires neither lytic growth or establishment of repression. Experiments using these methods demonstrate that λ integrative recombination is defective in *him*A mutants.

Second, two lines of evidence suggest that there is no appreciable interference with the transcription and translation of the *int* and/or *xis* gene in *him*A mutants. The first is derived from studies of a mutant Int protein, Int-h3, which is active under *him*A conditions (see accompanying paper, Miller et al., 1980). These studies show that *int*-h3 expression is regulated in a manner similar to *int*$^+$. Since Int-h3 is synthesized in *him*A$^-$ hosts, it follows that the *int* gene must be transcribed and translated in *him*A hosts in a relatively normal manner. Because Int-h3 requires Xis for excisive recombination under *him*A$^-$ conditions, the *xis* gene must also be expressed (Miller et al., 1980). The second line of evidence comes from in vitro studies which show that extracts of *him*A$^-$ lysogens of λ*int*-c contain normal specific activities of active Int protein (Miller et al., 1979). Thus failure to produce Int under *him*A$^-$ conditions cannot account for the defect in λ site-specific recombination.

Third, *Him*A$^-$ strains do not produce a negatively complementing protein, since bacteria diploid for the *him*A region which contain both a *him*A$^+$ and a *him*A$^-$ gene are phenotypically HimA$^+$. Collectively, these experiments indicate that the *him*A mutants are missing a function needed for the site-specific recombination event itself.

Although *him*A mutants were selected for the failure to integrate λ, phage with different Int and attachment-site specificities such as φ80 and P2 also fail to lysogenize these hosts efficiently (Miller et al., 1979). If the block also is at the level of integration, this implies that the *him*A gene product is also required for φ80 and P2 site-specific recombination. This indicates that the *him*A gene product plays a general role in site-specific recombination and is unlikely to be involved in nucleotide sequence recognition.

Lysogeny

As discussed above, there are alternative promoters for transcribing the *c*I (repressor) gene; one for establishment and the other for maintenance of expression. We have argued that *him*A interferes with the establishment mode of synthesis. Although we have not yet determined the manner in which the *him*A gene protein may regulate establishment synthesis, the fact that HimA is also required for integration could provide a convenient method for the host to coordinate the various processes involved in channeling λ towards either the lytic or lysogenic pathways. Thus, under conditions of limiting *him*A expression, both repression and integration could be reduced, resulting in channeling of the phage towards the lytic pathway. Maximal *him*A expression would favor both integration and repression, a situation promoting lysogeny. Thus the *him*A gene product may, like the λcII gene product (Katzir et al., 1976; Court et al., 1977) coordinate the two phases of lysogeny, repression and integration.

Growth of Bacteriophage Mu

The failure of Mu to propagate in *him*A$^-$ hosts due to a deficiency in site-specific recombination would certainly be consistent with current models for the Mu life-cycle. These models suggest that repeated integration of progeny Mu DNA molecules into the E. coli chromosome is a necessary step in Mu DNA encapsidation (Bukhari, 1976). Thus interference with this process would result in abortive lytic growth. Attractive as this explanation is, we cannot detect any difference in Mu integration in *him*A mutants by measurements of lysogeny frequency. This result does not exclude an effect on Mu integration per se, however, but only on integration that leads to lysogeny. Assuming that MuX excision mimics some essential feature of the Mu growth cycle, a lack of this type of recombination in *him*A$^-$ strains could account for defective Mu growth.

Several observations suggest that lack of Mu site-specific recombination is not the sole cause of the Mu growth defect. In contrast to wild-type bacteria which are efficiently killed by either Mu infection or induction, *him*A$^-$ bacteria exhibit nearly complete survival following initiation of growth by either method. Thus high-level expression of Mu-encoded functions that would normally result in host killing, Mu DNA replication, and maturation probably does not occur in *him*A$^-$ hosts. Consistent with this contention is the finding that less than one round of Mu DNA replication occurs in *him*A$^-$ bacteria infected with Mu (M. Pato, personal communication). Since *him*A$^-$ bacteria are lysogenized normally by Mu, and *him*A(mu) lysogens are immune to Mu*nu*A, the genes responsible for establishment and maintenance of both the Mu repressor and the Mu-encoded integration protein must be productively expressed. Indeed, under conditions of spontaneous induction, mu must be capable of expressing and utilizing all the gene products necessary to produce a viable phage.

Reversion of Insertion Mutations

We have described the effect of *him*A mutations on the reversion of insertion-induced mutations for three insertion elements; MuX, Tn10 and Tn5. The reduction in reversion frequencies for MuX is quite dramatic. The defect in reversion of Tn5 and Tn10 induced mutations, however, is much less extensive. We have recently isolated new *him*A mutants, some of which appear to be deletions of the *him*A gene, that reduce the reversion rate of both Tn5- and Tn10-induced mutation by approximately three orders of magnitude. In addition, these new *him*A mutants are as defective in excising a chromosomally located element as in excising one that is episomally located (H. Miller, unpublished observations). Thus the small reductions in reversion on Tn10 mutations seen in strains carrying the *him*A42 mutation probably reflect the leakiness of his particular mutation with regard to insertion mutation reversion.

The *him*A Gene Product

The studies reported here leave open the question of the role of *him*A gene product in site-specific recombination as well as in other processes. The *him*A protein could act by directly participating in each of the processes, or indirectly by regulating the synthesis of several factors.

Recent experiments clarify the role of the *him*A gene product in λ site-specific recombination. The product of the *him*A gene has been identified by analyzing proteins labeled in ultraviolet-irradiated cells infected with transducing phage carrying the *him*A gene. (H. Miller, manuscript in preparation). Moreover, *him*A protein has been shown to be a major component of purified integration host factor active in in vitro λ integrative recombination (H. Miller and H. Nash, manuscript in preparation). This result suggests that *him*A protein plays a direct role in λ site-specific recombination. It is probable, therefore, that *him*A protein participates directly in all the processes affected by *him*A mutations. Considering the nature of these processes, one role of *him*A protein might be to facilitate the interaction between DNA and protein. This would be consistent with the *him*A protein participating in both site-specific recombination and gene regulation. The fact that *him*A protein is required for integration of phage with different *att* site specificities suggests that it is not involved in determining the specificity of DNA site selection.

Experimental Procedures

Media

TB contains 1% Difco tryptone broth and 0.25% NaCl. LB is TB + 0.5% yeast extract. LBMM is LB + 10 mM $MgSO_4$ + 0.2% maltose. Minimal media contained (per l) 6 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 0.25 g $MgSO_4$-$7H_2O$ and 0.4% carbon source. All of the above plate media was solidified with 1% Difco agar. Gal-TTC plates are TB plates + 1% D-galactose and 0.0025% 2,3,5-triphenyltetrazolium chloride from Eastman (Enquist and Weisberg, 1976). TCMG plates contain 1% BBL trypticase, 0.85% Difco agar, 0.5% NaCl and 10 mM $MgSO_4$. TE is 10 mM Tris (pH 7.4) + 10 mM EDTA. EBMO plates are described in Gottesman and Yarmolinsky (1968). MacConkey plates for assaying sugar fermentation contained Difco MacConkey base and 1% sugar. Amino acid supplements to minimal media were at 10 μg/ml.

Frequency of Lysogeny

The frequency of lysogeny was measured using essentially the method of Gottesman and Yarmolinsky (1968). A fresh culture of the bacteria in LBMM was infected with phage at an moi of 5. After a 10 min adsorption period, dilutions of the infected cells were spread on TB plates and on EMBO plates seeded with 10^9 clear-plaque counterselecting phage of the same immunity as the test phage. Using this method, lysogens appear as normal pink colonies on the EMBO plates, whereas nonlysogens or abortive lysogens appear as irregular, dark purple colonies. The ratio of lysogens to total surviving cells is taken as a quantitative measure of lysogeny.

Red Plaque Assay for Int and Xis

Phages were plated on lawns of derivatives of K387 on Gal-TCC plates as described by Enquist and Weisberg (1976). On this lawn, int^+-xis^+phages give plaques with red centers. Phages that are int^- or xis^- give plaques with colorless centers. The red-centered plaques result from the Int-Xis-promoted excision of a cryptic λ prophage inserted in the *galT* gene of K387. The excision of this cryptic prophage results in the formation of gal^+ lysogens which grow in the center of the plaque, ferment the galactose, and reduce the tetrazolium dye to a red derivative.

Mating and Transduction

Bacterial conjugal mating and P1 transduction were performed using standard procedures as outlined in Miller (1972).

Construction of *him*A⁻ Strains

The *him*A alleles were introduced into strains by either specialized or generalized transduction. Specialized transduction was effected using variants of a λ transducing phage that carries the *him*A gene (see Figure 1). The *him*A allele could be transferred by generalized transduction using phage P1, if the recipient was pps^- (growth on lactate) (Hansen and Juni, 1974). In this case, the strain was transduced to pps^+ with P1 from the $himA^-$ donor strain and the transductants screened for the $himA^-$ phenotype.

Scoring for the *him*A⁻ Phenotype

The *him*A phenotype was scored as the failure to plate phage Muc25, λcI857 *cin*-1 at 32°C or by the inability to integrate λ as described above.

Acknowledgments

The authors thank Drs. M. Howe, L. Enquist, D. Court, M. Gottesman, S. Adhya, A. Kikuchi, R. Weisberg and H. Nash for helpful discussion. We also acknowledge the excellent technical help of Sherry Parsons, as well as the patience and skill of Stacy Stachler and Brenda Barnes in preparing the manuscript. This research was supported by grants from the NIH to D. Friedman and from the American Cancer Society to the University of Michigan.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 15, 1979; revised March 31, 1980

References

Berg, D. E. (1976). Insertion and excision of the transposable kanamycin resistant determinant Tn5. In DNA Insertion Elements, Plasmids and Episomes. A. Bukhari, J. Shapiro and S. Adhya, eds. (New York: Cold Spring Harbor Laboratory), pp. 205–212.

Berg, D., Davies, J., Allet, B. and Rochaix, J. D. (1975). Transposition of R factor genes to bacteriophage λ. Proc. Nat. Acad. Sci. USA *72*, 3628-3632.

Bertani, L. (1970). Split operon control of a prophage gene. Proc. Nat. Acad. Sci. USA *65*, 331-336.

Bukhari, A. I. (1975). Reversal of mutator phage Mu integration. J. Mol. Biol. *96*, 87-99.

Bukhari, A. I. (1976). Bacteriophage Mu as a transposition element. Ann. Rev. Genet. *10*, 389-412.

Campbell, A. (1962). Episomes. Adv. Genet. *11*, 101-145.

Court, D., Adhya, S., Nash, H. and Enquist, L. (1977). The phage λ integration protein (Int) is subject to control by the cII and cIII gene products. In DNA Insertion Elements, Plasmids and Episomes, A. Bukhari, J. Shapiro and S. Adhya, eds. (New York: Cold Spring Harbor Laboratory), pp. 389-394.

Echols, H., Gingery, R. and Moore, L. (1968). Integrative recombination function of bacteriophage λ: evidence for a site-specific recombination enzyme. J. Mol. Biol. *34*, 251-260.

Eisen, H., Brachet, P., Pereira da Silva, L. and Jacob, F. (1970). Regulation of repressor expression in λ. Proc. Nat. Acad. Sci. USA *66*, 855-862.

Enquist, L. W. and Weisberg, R. A. (1976). The red plaque test: a rapid method for identification of excision defective variants of bacteriophage lambda. Virology *72*, 147-153.

Fowler, R. G., Degnen, G. E. and Cox, E. C. (1974). Mutational specificity of a conditional *Escherichia coli* mutator, *mut*D5. Mol. Gen. Genet. *133*, 179-191.

Gottesman, M. E. and Yarmolinsky, M. B. (1968). Integration-negative mutants of bacteriophage lambda. J. Mol. Biol. *31*, 487-505.

Guarneros, G. and Echols, H. (1970). New mutants of bacteriophage λ with a specific defect in excision from the host chromosome. J. Mol. Biol. *47*, 565-574.

Hansen, E. J. and Juni, E. (1974). Two routes for synthesis of phosphoenol pyruvate from c4-dicarboxylic acids in *Escherichia coli*. Biochem. Biophys. Res. Commun. *59*, 1204-1210.

Hennecke, H., Springer, M. and Bock, A. (1977a). A specialized transducing phage carrying the *E. coli* genes for phenylalanyl-tRNA synthetase. Mol. Gen. Genet. *152*, 205-210.

Hennecke, H., Bock, A., Thomale, J. and Nass, G. (1977b). Threonyl-transfer ribonucleic acid synthetase from *E. coli*: subunit structure and genetic analysis of the structural gene by means of a mutated enzyme and of a specialized transducing bacteriophage. J. Bacteriol. *131*, 943-950.

Howe, M. and Bade, E. (1975). Molecular biology of bacteriophage Mu. Science *190*, 624-632.

Katzir, N., Oppenheim, A., Belfort, M. and Oppenheim, A. B. (1976). Activation of the lambda *int* gene by the cII and cIII gene products. Virology *74*, 324-331.

Kleckner, N. (1977). Translocatable elements in procaryotes. Cell *11*, 11-23.

Kleckner, N., Chan, R., Tye, B. D. and Botstein, D. (1975). Mutagenesis by insertion of a drug resistance element carrying an inverted repetition. J. Mol. Biol. *97*, 561-575.

Kourilsky, P., Perricaudet, M., Gros, D., Garapin, A., Gottesman, M., Fritsch, A. and Tiollais, P. (1978). Description and properties of some Eco RI vectors derived from bacteriophage. Biochimie *60*, 183-187.

Landy, A. and Ross, W. (1977). Viral integration and excision: structure of the lambda *att* sites. Science *197*, 1147-1160.

Miller, H. I. and Friedman, D. I. (1977). Isolation of *Escherichia coli* mutants unable to support lambda integrative recombination. In DNA Insertion Elements, Plasmids and Episomes, A. Bukhari, J. Shapiro and S. Adhya, eds. (New York: Cold Spring Harbor Laboratory), pp. 349-356.

Miller, H. I., Kikuchi, A., Nash, H. A., Weisberg, R. A. and Friedman, D. I. (1979). Site-specific recombination of bacteriophage λ: the role of host gene products. Cold Spring Harbor Symp. Quant. Biol. *43*, 1121-1126.

Miller, H. I., Mozola, M. A. and Friedman, D. I. (1980). *Int*-h: an *int* mutation of phage λ that enhances site-specific recombination. Cell: *20*, 721-729.

Miller, J. H. (1972). Experiments in molecular genetics. (New York: Cold Spring Harbor Laboratory).

Mizuuchi, K. and Nash, H. A. (1976). Restriction assay for integrative recombination of bacteriophage λ DNA *in vitro*: requirement for closed circular DNA substrate. Prac. Nat. Acad. Sci. USA *73*, 3524-3528.

Nash, H. A. (1974). λ *att*B-*att*P, a λ derivative containing both sites involved in integrative recombination. Virology *57*, 207-216.

Nash, H. A. (1977). Integration and excision of bacteriophage λ. Curr. Topics Microbiol. Immunol. *78*, 171-199.

Parkinson, J. S. and Huskey, R. J. (1971). Deletion mutants of bacteriophage lamda. I. Isolation and initial characterization. J. Mol. Biol. *56*, 369-384.

Ptashne, M., Bachman, K., Humagon, M. Z., Jeffrey, A., Maurer, R., Meyer, B. and Asuer, R. T. (1976). Autoregulation and function of a repressor in bacteriophage lambda. Science *194*, 156-161.

Reichardt, L. and Kaiser, A. D. (1971). Control of λ repressor synthesis. Proc. Nat. Acad. Sci. USA *68*, 2185-2189.

Shimada, K. and Campbell, A. (1974). Int-constitutive mutants of bacteriophage lambda. Proc. Nat.. Acad. Sci. USA *71*, 237-241.

Shimada, K., Weisberg, R. A. and Gottesman, M. E. (1972). Prophage lambda at unusual chromosomal locations. I. Location of the secondary attachment sites and the properties of the lysogens. J. Mol. Biol. *63*, 483-503.

Shimada, K., Weisberg, R. A. and Gottesman, M. E. (1973). Prophage lambda at unusual chromosomal locations. II. Mutations induced by bacteriophage lambda in *Escherichia coli* K12. J. Mol. Biol. *80*, 297-314.

Shulman, M. and Gottesman, M. E. (1971). Lambda *att*^{2}: a transducing phage capable of intramolecular *int-xis* promoted recombination. In The Bacteriophage Lambda, A. D. Hershey, ed. (New York: Cold Spring Harbor Laboratory), pp. 477-487.

Shulman, M. and Gottesman, M. E. (1973). Attachment site mutants of bacteriophage lambda. J. Mol. Biol. *81*, 461-482.

Signer, E., Echols, H., Weil, J., Radding, C., Shulman, M., Moore, L. and Manly, K. (1968). The general recombination system of bacteriophage λ. Cold Spring Harbor Symp. Quant. Biol. *33*, 711-719.

Springer, M., Graffe, M. and Hennecke, H. (1977). Specialized transducing phage for the initiation factor IF3 gene in *E. coli*. Proc. Nat. Acad. Sci. USA *74*, 3970-3974.

Starlinger, P. and Saedler, H. (1972). Insertion mutations in microorganisms. Biochimie *54*, 177-185.

Starlinger, P. and Saedler, H. (1976). IS-elements in microorganisms. Curr. Topics Microbiol. Immunol. *75*, 111-152.

Taylor, A. L. (1963). Bacteriophage-induced mutations in *Escherichia coli*. Proc. Natl. Acad. Sci. *50*, 1043-1051.

Weisberg, R. and Gallant, J. (1967). Dual function of the λ prophage repressor. J. Mol. Biol. *25*, 537-544.

Weisberg, R. A., Gottesman, S. and Gottesman, M. E. (1977). Bacteriophage λ: the lysogenic pathway. In Comprehensive Virology, *8*, Fraenkel-Conrat and R. Wagner, eds. (New York: Plenum Press), pp. 197-258.

Williams, J. G. K., Wulff, D. L. and Nash, N. A. (1977). A mutant of *Escherichia coli* deficient in a host function required for phage lambda integration and excision. In DNA Insertion Element, Plasmids and Episomes. A. Bukhari, J. Shapiro and S. Adhya, eds. (New York: Cold Spring Harbor Laboratory), pp. 357-361.

Zissler, J. (1967). Integration-negative (*int*) mutants of phage λ. Virology *31*, 189.

Zissler, J., Signer, E. and Schaefer, F., (1971). The role of recombination in growth of bacteriophage lambda. I. The gamma gene. In The Bacteriophage Lambda, A. D. Hershey, ed. (New York: Cold Spring Harbor Laboratory), pp. 455-468.