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int-h: an *int* Mutation of Phage λ That Enhances Site-Specific Recombination

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Summary

The mutation *int*-h3 maps in the *int* gene of coliphage λ and results in the synthesis of an integrase with enhanced activity, which is manifested by an ability to support λ site-specific recombination relatively efficiently under conditions where the wild-type integrase functions inefficiently. The level of site-specific recombination seen in the presence of the *int*$^+$ integrase in *himA*$^-$ hosts is greatly reduced, as measured by lysogen formation, intramolecular site-specific integration and excision, and excision of a cryptic λ prophage. In contrast, the *int*-h3 integrase shows relatively high levels of activities under these conditions. *Int*-h3 is also more active in other host mutants (*him*B and *hip*) that reduce λ site-specific recombination. In the absence of the normal *att*B site, the frequency of lysogen formation (at secondary sites) by λ *int*$^+$ is reduced 200 fold. Although λ *int*-h3 will integrate preferentially at the *att*B site if it is present, the mutant phage forms lysogens at a high frequency in *att*B-deleted hosts. λ *int*-h3 requires *him*A function for integration at secondary sites. The fact that the *int*-h3 integrase uses the same *att* sites as well as the same host functions as the *int*$^+$ integrase suggests that the mutation results in a quantitative rather than a qualitative change in integrase activity; that is, the *int*-h3 integrase is more active. The mutant integrase supports site-specific recombination with *att* sites that carry the *att*24 mutation. We propose that the *int*-h3 integrase is endowed with an enhanced ability to recognize *att* sequences, including some that are not effectively recognized by wild-type integrase.

Introduction

The recombinational event that results in the integration of bacteriophage λ into the E. coli chromosome requires the participation of phage- and host-encoded components (see Figure 1) (reviewed by Nash, 1977; Miller et al., 1979). As first proposed by Campbell (1962), a reciprocal site-specific recombination occurs between two unique nucleotide sequences, called attachment or *att* sites, located on both the phage (*att*P) and bacterial (*att*B) chromosomes. Upon integrations, two composite *att* sites are generated flanking the left (*att*L) and right (*att*R) prophage ends (Gottesman and Weisberg, 1971). Each *att* site contains an identical 15 nucleotide "common core" sequence (designated "0") which separates the unique elements P, P′, B and B′ (Shulman and Gottesman, 1973; Landy and Ross, 1977). Thus *att*P can be written as POP′, *att*L as BOP′, and so on. The breakage and rejoining of DNA strands occurs within the core sequence.

λ integration can occur in the absence of *att*B. The frequency of integration in hosts lacking *att*B is reduced 200 fold (Shimada, Weisberg and Gottesman, 1972), however, and under these conditions occurs at a limited array of secondary sites. Analysis of several of these sites suggests a sequence relationship with the common core (Bidwell and Landy, 1979; Christie and Platt, 1979).

Integration (*att*P × *att*B) depends on Int protein (Zissler, 1967; Gottesman and Yarmolinsky, 1968b), while excision (*att*L × *att*R) requires, in addition to Int, Xis protein (Guarneros and Echols, 1970; Kaiser and Masuda, 1970). These two products are encoded, by the phage *int* and *xis* genes, respectively. At least one host component is also necessary for both integration and excision (indicated as Him, for *h*ost *i*ntegration *m*ediator). In the accompanying paper (Miller and Friedman, 1980), we describe the properties of bacterial strains lacking a host protein required for λ integration. These strains are defective for λ site-specific recombination due to mutations in the E. coli *him*A gene (Miller and Friedman, 1977). Mutations in several other unlinked loci, such as *hip*, *him*B and *him*C, also exhibit λ integration-defective phenotypes (Miller et al., 1979).

Since Int proteins from related bacteriophages are not active for recombining the λ *att* sites (Signer and Beckwith, 1966; Gottesman and Yarmolinsky, 1968a), the specificity for nucleotide sequence recognition appears to reside in the Int protein. The opposite is true for the host components identified so far, since they are required for integration of phage with differing Int and *att* site specificities (Miller et al., 1979).

We have selected λ mutants able to integrate efficiently under conditions where the host integration activity is reduced (*him*A$^-$ conditions). We now report the isolation and characterization of one such mutation in the Int structural gene, *int*-h3. This mutation results in the production of an Int protein that is active in a variety of host mutants normally defective in λ site-specific recombination. The properties of λ *int*-h3 suggest that the host component aids Int protein in recognizing the attachment site.

Results

Isolation and Initial Characterization of λ3

Mutants of λ able to overcome the block imposed on site-specific recombination by the *him*A42 mutation

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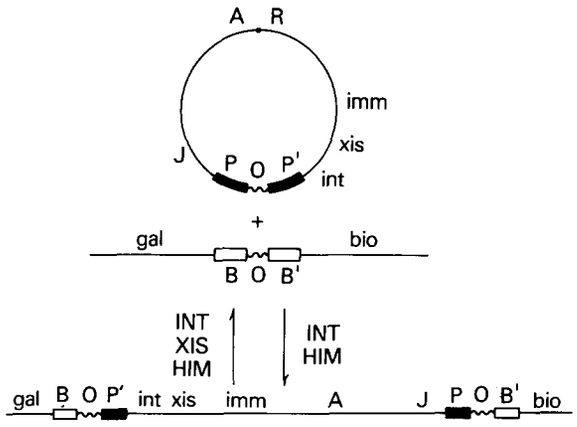

Figure 1. Integration and Excision of Bacteriophage λ

Representative λ genetic markers are indicated; J, A and R (tail, head and lysis genes), *imm* (regulation genes including cI repressor gene), and *int* and *xis* (site-specific recombination genes). The wavy line below "O" indicates the common core. The four additional constituents of the *att* sites are labeled as P, P′, B and B′.

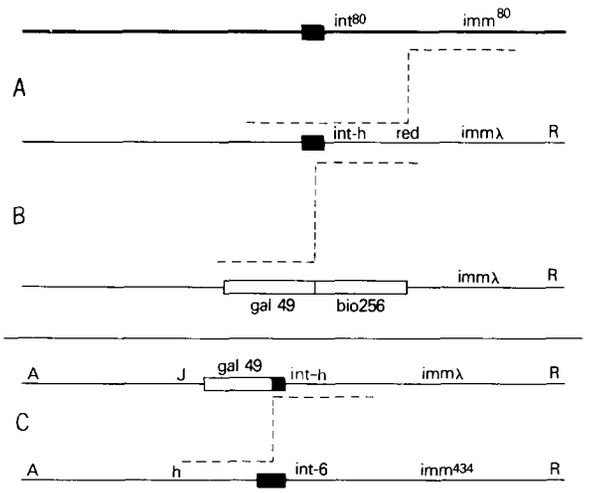

Figure 2. Construction of Recombinants Carrying *int*-h3

(A) λ*imm*80*int*-h3: Phage λ80 was crossed with λ3 and λ*imm*80*int*-h3 recombinants were selected by plating dilutions of the cross on Gal-TTC plates seeded with K600, a λ lysogen of the red plaque strain K387. After overnight incubation, phage from red plaques were tested for the Int-h phenotype and φ80 immunity.

(B) λ*int*-h3*gal*49: λ*gal*49*bio*256 was crossed with λ3. Dilutions were plated on K100 (a *rec*A strain) to select against phage carrying *bio*256 (Zissler, Signer and Schaefer, 1971) at 41°C to select against λ3. Individual plaques were isolated and the phages were tested for *gal*49 by determining whether K545 (*gal*E⁻) can be transduced to galactose resistance (Sundaraarajan, Rapin and Kalckar, 1962).

(C) λ*int*-h3*attP*⁺: λ*int*-h3*gal*49 (from B) was crossed with λ*imm*434c-int6 *h*, and dilutions were plated on K889, a lysogen of λ*imm*434 that selects for the *h* marker. Phages purified from the resulting plaques were tested for the *int*-h3 phenotype and for loss of *gal* genes as in B.

Black boxes represent the phage *att* site.

(Miller and Friedman, 1980) were obtained as outlined in Experimental Procedures. One mutant, λ3, which formed a red plaque on a *him*A⁻ derivative of the red plaque strain (see Experimental Procedures and Enquist and Weisberg, 1976), was selected for further characterization.

The λ3 mutant exhibited other altered characteristics: temperature-sensitive growth, a reduced ability to form stable lysogens, and insensitivity to treatment with chelating agents (a characteristic indicative of a smaller than normal genome; Parkinson and Huskey, 1971). However, these characteristics could be crossed out, as described in Figures 2B and 2C, without influencing the enhanced effect on integrative recombination.

Analysis of the recombinants formed between λ3 and two phages that are partially nonhomologous with λ established that the mutation responsible for the enhancement of site-specific recombinational activity maps in the vicinity of the *int* gene. The phages used in the cross are the related lambdoid phage φ80 and a λ derivative that carries bacterial genes substituting for some phage genes, λ*gal*49-*bio*256. The structures of these phages, as well as the recombination events, are diagrammed in Figures 2A and 2B. It should be noted that neither phage carries the *int* allele of λ. φ80 carries the *int* gene specific to that phage and λ*gal*49-*bio*256 carries biotin genes substituted for the *int* gene. The φ80 immunity region responsible for repressor synthesis and repressor binding specificity is in a segment of the genome nonhomologous with λ (Fiandt et al., 1971); that is, located to the right of the *int* gene and beginning within the *red* gene. The *gal-bio* phage carries a substituted segment which is not homologous to λ and which spans the regions immediately to the right and left of *att*, and thus the nonhomology includes the *int* gene.

First, consider the cross between φ80 and λ3 (Figure 2A). Recombinants that carry the immunity of φ80 and the integration specificity of λ all exhibit the *int*-h3 phenotype. This locates the *int*-h3 mutation to the left of the λ *red* gene and is consistent with the mutation mapping in the *int* gene region. Second, consider the cross between λ*gal*49-*bio*256 and λ3 (Figure 2B). Recombinants identified as carrying a λ*int* gene and capable of transducing the *gal*E marker all exhibited the *int*-h3 phenotype. This cross locates the mutation to the right of *att*. The results of these crosses, then, locate the *int*-h3 mutation in the region to the left of the *red* gene and to the right of *att*.

The *int*-3 mutation was located within the *int* gene by deletion mapping. A φ80 derivative carrying the immunity of φ80 and the *int*-h3 mutation (from the cross in Figure 2A) was crossed with two λ variants, one carrying an *int* deletion and the other a substitution of a part of the *int* gene. The structure of the phage and the details of these crosses are schematically represented in Figure 3. As shown, λ*b*2043 carries a small deletion, while λ*bio*2037 replaces most of the *int* gene with substituted bacterial genes (Enquist and Weisberg, 1977). Recombinants with the immunity of λ and an active *int* gene were tested to

determine whether they carried the *int*-h3 mutation. If the *int*-h3 mutation is located within the region defined by a given deletion, recombinants that carry immunity λ and display Int λ activity would necessarily have to carry the *int*-h3 mutation. On the other hand, if the deletion does not cover the *int*-h3 mutation, then recombinants should be formed that have the immunity of λ and display Int λ activity, but do not necessarily have the *int*-h3 mutation. Characterization of the recombinants formed between λ*b*2043 and φ80*int*-h3 (Figure 3A) revealed that 17% of the recombinants that displayed Int activity were not carrying the *int*-h3 mutation. For comparison, an analagous φ80 derivative that has the *int am*29 mutation was used in a similar cross. Recombinants with λ immunity that express Int activity in a *su*III$^+$ host were isolated. Subsequent tests revealed that 26% of these recombinants had a wild-type *int* gene (74% had the *int am*29 mutation). A comparison of recombination frequencies places the *int*-h3 mutation to the left of *int am*29. Consistent with this conclusion is the fact that all of 1000 analyzed λ recombinants expressing λ Int activity formed by the cross between λ *bio*2037 (a phage missing most of the *int* gene) and φ80*int*-h3 (Figure 3B) displayed the *int*-h3 phenotype. This result suggests that *int*-h3 maps under this deletion. In these mapping experiments we have assumed that the Int-h phenotype results from a single mutation. If this is not the case, our crosses would map the right-most mutation of the mutations responsible for this phenotype.

These experiments locate the mutation(s) responsible for the *int*-h3 activity between the right endpoints of the *b*2043 deletion and the *int am*29 mutation; that is, within the *int* gene. This mapping therefore legitimizes our initial nomenclature for the mutation as *int*-h3. In all the following experiments the λ*int*-h phage used will be the recombinant generated by the cross shown in Figure 2C.

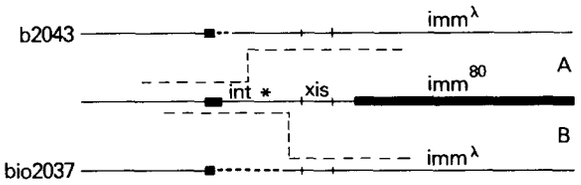

Figure 3. Deletion Mapping of the *int*-h3 Mutation

Phage crosses were performed between λ*imm*80*int*-h3 and either λ*b*2043 orλ*bio*2037, while λ*int am*29 was crossed only with λ*b*2043. For mapping *int*-h3, dilutions of the cross were plated on K734 (a φ80 lysogen of the red plaque strain) on Gal-TTC plates and incubated at 32°C. Bacteria on the plate were killed by inverting the plate over filter paper saturated with chloroform. The centers of red plaques were picked with a sterile toothpick and the phages were tested on a lawn of K748 (a φ80 lysogen of the *him*A red plaque strain) on Gal-TTC plates. For mapping the *int am*29 mutation, dilutions of the cross were plated on K397 (a φ80 lysogen of a *su*III$^+$ red plaque strain). Plaques scored as Int$^+$ on the Su$^+$ strain were tested on the Su$^-$ strain K734.

In the cross with λ*b*2043, 17% of the *imm*λ phage which expressed active Int had lost the *int*-h3 mutation, whereas 26% had lost the *int am*29 mutation. In the case of λ*bio*2037, none out of 1000 such recombinants lost the *int*-h3 mutation.

The heavy dashed line gives an indication of the extent of the deletions into the *int* gene. The region where the *int*-h3 and *int am*29 mutations are located is indicated by (*).

Activity of the *int*-h3 Integrase in the *him*A$^-$ Host

The activity of the *int*-h3 integrase in *him*A$^-$ hosts was measured using a variety of tests of λ site-specific recombination. In each case it could be demonstrated that the *int*-h3 integrase, unlike the wild-type, supported site-specific recombination in a *him*A$^-$ background. The results are presented in Tables 1 and 2.

Stable lysogen formation, an indirect measure of site-specific recombination, was used to study integration in the *him*A mutants (see Table 1). Under the conditions that we used to test for lysogen formation, the control *him*$^+$ bacteria are efficiently lysogenized by λ *int*$^+$; approximately 90% of the bacteria surviving infection are lysogenized. In the *him*A42 host, only 0.01% of the bacteria surviving infection with λ*int*$^+$ are lysogens. In contrast, approximately 30% of the *him*A$^-$ bacteria surviving infection with λ*int*-h3 are lysogens. This significant level of lysogen formation by λ*int*-h3 in the *him*A$^-$ hosts is reflected in tests that more directly measure site-specific recombination.

In the case of integrative recombination, co-infection of a *him*$^+$ host with λ*att*B-*att*P (Nash, 1974) and a phage that supplies wild-type integrase results in 34% of the λ*att*B-*att*P phage losing the bacterial genes (Table 2); that is, a 34% conversion rate. This conversion is increased to 70% when the second phage carries the *int*-h3 mutation. In analogous experiments with *him*A$^-$ hosts, there is no observable conversion of λ*att*B-*att*P in the presence of the *int*$^+$ product (0.1%); this being no greater than the conversion seen

Table 1. Frequency of Lysogeny by λ*int*-h3

		% Lysogeny	
Bacteria	Genotype	*int*$^+$	*int*-h3
K37	++	>90	>90
K985	Δ*att*B	0.5	30
K936	*him*A42	$<10^{-2}$	34
K1020	*him*A42Δ*att*B	$<10^{-2}$	5×10^{-2}

Bacteria grown in LBMM at 32°C to a concentration of 2×10^8/ml were pelleted and resuspended in 10 mM $MgSO_4$. The cells were infected with either λcI857*int*$^+$ or λcI857 *int*-h3 *h* at an moi of 10. After a 10 min adsorption, dilutions were spread on EMBO plates and incubated overnight at 32°C. Lysogens were scored as colonies immune to λ*c*I$^-$, but sensitive to λ*imm*434 in a cross-streak test. For strains displaying a high frequency of lysogeny, the frequency was determined by examining individual surviving colonies for λ immunity. For strains exhibiting a low frequency of lysogeny, the dilutions of the infected cells were spread on EMBO plates seeded with 10^9 λ*c*I$^-$*b*2 and survivors were tested for immunity as above. Frequencies are expressed as (immune lysogens per total surviving cells) × 100.

Table 2. Integrative and Excisive Recombination Catalyzed by λ*int*-h3

		% Recombination			
		*att*B-*att*P[a]		*att*L-*att*R[b]	
Strain	Genotype	*int*$^+$	*int*-h3	*int*$^+$	*int*-h3
K37	+	34	70	68	70
K648	*him*A*am*79	0.2	8	3	34
K634	*him*A42	0.1	9	2	23
K770	*hip*157	0.7	12	nt	nt

The details of procedure are given in Miller and Friedman (1980). Numbers are percent *h*$^+$ EDTA resistant phage after single cycle growth of either (a) λ*imm*21*c int*2 *xis*1 *red*114 *att*B-*att*P (λ*att*B-*att*P) or (b) λ*imm*21*c int*2 *xis*1 *red*114 *att*L-*att*R (λ*att*L-*att*R). The moi was 1. Int and Xis was supplied by either λcI857 (λ*int*$^+$) or λcI857*int*-h3 *h* (λ*int*-h3) at an moi of 5. (nt) not tested.

in the absence of a co-infecting, Int-supplying phage. The enhanced activity of the *int*-h3 integrase is clearly evidenced when λ*int*-h3 is used as the co-infecting phage. Breakdown of λ*att*B-*att*P is 8–9% in both the *him*A*am*79 and the *him*A42 hosts.

The level of excisive recombination observed using λ*att*L-*att*R (Shulman and Gottesman, 1973) for a co-infecting *int*$^+$*xis*$^+$ phage is 20–30 fold lower in a *him*A$^-$ mutant in comparison to that observed in a *him*$^+$ host. The enhanced activity of the *int*-h3 integrase is evidenced by the observation of almost normal levels of excisive recombination in a *him*A$^-$ host when the co-infecting Int-supplying phage carries the *int*-h3 mutation. In this case the reduction in the conversion reaction is not 20 fold but is only a modest 2–3 fold.

Activity of *int*-h3 in Other Integration-Defective Host Mutants

In addition to the locus defined by *him*A$^-$ mutations, two other bacterial loci have been shown to be involved in λ site-specific recombination. These loci are *him*B, mapping at minute 81, and *hip*, mapping at minute 19, on the standard E. coli genetic map (Miller et al., 1979; Miller and Friedman, 1980; A. Kikuchi and R. A. Weisberg, personal communication). The studies discussed below show that the *int*-h3 integrase facilitates site-specific recombination in hosts carrying *him* mutations at these other loci.

Mapping studies (Miller et al., 1979) locate the *him* B114 mutation directly in the region of the *cou*^{R} gene (Ryan and Wells, 1976). This gene has been shown to encode for one of the subunits of DNA gyrase, an enzyme that puts superhelical turns in DNA (Gellert et al., 1976). An effect of the *him*B mutation on DNA gyrase activity has been demonstrated (M. Gellert, personal communication). Although DNA gyrase has been implicated in site-specific recombination (Mizuuchi, Gellert and Nash, 1978), no other mutation associated with this locus has been shown to influence the site-specific recombination reaction. The *him*B114 mutation causes only a modest reduction in the level of λ site-specific recombination catalyzed by the wild-type integrase. This inhibitory effect can only be observed using a red plaque derivative carrying the *him*B114 mutation. When a *him*B114 red plaque host is used as the bacterial lawn, λ*int*$^+$ forms colorless plaques under the standard plating procedures used for the test. On the other hand, *int*-h3 derivatives evidence enhanced Int activity, for they form the usual type of red plaque on the *him*B114 strain. Other, more sensitive assays of integrase activity fail to differentiate between *him*$^+$ and *him*B114 (data not shown).

The phenotype of the *hip* mutant more closely resembles that of *him*A mutants (Miller et al., 1979; A. Kikuchi and R. A. Weisberg, personal communication). Like *him*A mutations, the *hip* mutation causes a marked reduction in λ site-specific recombination as measured by a variety of tests. We have tested the activity of *int*-h3 in a *hip*$^-$ host (K770) and find that *int*-h3 can also catalyze integrative recombination in the *hip*$^-$ host (Table 2). In addition, λ*int*-h3 forms a red plaque on a *hip*$^-$ red plaque strain (K1039).

These experiments establish that the *int*-h3 integrase is active in a variety of cases where the host is defective in the expression of a factor necessary for site-specific recombination. However, the results of these experiments do not permit us to determine whether the *int*-h3 integrase is able to function independently of these host factors or, alternatively, whether the altered integrase can function effectively with reduced levels of active host factors. In regard to the latter point, it is probable that the various host mutants are leaky and thus express a level of activity that is sufficient to permit the *int*-h3 integrase to be active. Under special conditions that will be discussed in the next section, the *int*-h3 integrase does require *him*A product for activity.

Integration of λ*int*-h3 at Primary and Secondary *att* Sites

We next determined whether the extraordinary activity of the *int*-h3 integrase is limited to hosts deficient in the expression of factors necessary for site-specific recombination or whether the altered integrase is also more active at catalyzing recombination at sites varying from those normally used by integrase. This question follows from the fact that λ site-specific recombination requires the appropriate *att* sites; for example, stable lysogen formation by λ is reduced 200 fold in a bacterial host whose chromosome in deleted for *att*B (Gottesman and Yarmolinsky, 1968a, 1968b). Shimada et al. (1972) and Shimada, Weisberg and Gottesman (1973) concluded that in the absence of *att*B, a series of unique secondary sites are available for λ integration. However, the low frequency of lysogeny in *att*B-deleted hosts by λ*int*$^+$ clearly demon-

strates that integrative recombination catalyzed at secondary sites by wild-type Int protein is very inefficient. These characteristics suggested that lysogeny in an *att*B-deleted host should offer another modality for studying the extended activity of the *int*-h3 integrase.

The enhanced activity of the *int*-h3 integrase is indeed reflected by an increased activity at secondary *att* sites. The results of experiments demonstrating this point are shown in Table 1. It can be seen that λ*int*-h3 shows only a slight reduction in lysogen formation in the *att* B-deleted host in comparison to the 200 fold reduction seen in the case of λ*int*$^+$.

Although the *int*-h3 integrase is highly active in supporting integration at secondary sites, experiments with a *him*A- and *att*-deleted strain (K1020) revealed, surprisingly, that the *int*-h3 integrase requires the *him*A gene product in order to support integration at secondary sites. As shown in Table 1, stable lysogen formation by λ*int*-h3 in the *him*A- and *att*-deleted strain is severely reduced. This finding indicates that at some level the *int*-h3 integrase requires host factors for activity. The implications of this finding will be more fully developed in the Discussion.

Location of *att* Sites Used by the *int*-h3 Integrase

The finding that the *int*-h3 integrase efficiently supports integration at secondary *att* sites led us to consider the nature of the *att* site preferred by this integrase. First, we determined whether λ*int*-h3 integrates preferentially at *att*B. Second, we determined whether the enhanced activity at secondary sites reflects an increased affinity either for the usual secondary *att* sites or for different sites than those used by the wild-type integrase. The experiments outlined below are consistent with the premise that the *int*-h3 integrase acts at the same *att* sites as the wild-type protein and shows, within the limits of our experiments, the same hierarchical order of site preference.

Integration of λ at the primary *att*B site can be demonstrated using a simple test based on prophage-dependent expression of galactose genes, a phenomenon called "escape synthesis." Details of this test are outlined in Experimental Procedures.

Using this test, we find that all of 40 independently isolated λ*int*-h3 lysogens contained prophages mapping in the *att*B region. All these lysogens were apparently multiple lysogens, since they were immune to λ*c*I$^-$c17 (Shimada et al., 1972). We have not determined whether, in addition to prophage at *att*B, a secondary site prophage was also present. These results, coupled with the observation that when *att*B is present lysogeny by λ*int*-h3 is increased 3 fold (Table 1), lead us to conclude that *att*B is the preferred site for λ*int*-h3 integration.

The identification of secondary sites of integration is by necessity less definitive, since only a fraction can be genetically defined. Shimada et al. (1973) found that 3% of the lysogens formed by λ*int*$^+$ in an *att*B-deleted host were auxotrophic. The vast majority of these were Pro$^-$. We find similar results for λ*int*-h3. Out of 150 lysogens screened there were three auxotrophs, and all were Pro$^-$. One note of caution is in order. Since we have not genetically determined the *pro* site used by λ*int*-h3, it cannot be categorically identified as identical to the *pro* site used by λ*int*$^+$. Although the precise sites of integration used by λ*int*-h3 in the nonauxotrophic lysogens have not been identified, the observation of identical frequencies of auxotroph formation, coupled with the finding that in both cases the majority of auxotrophs formed are Pro$^-$, is consistent with the idea that the sites of secondary integration used by λ*int*-h3 are the same as those used by λ*int*$^+$.

Action of *int*-h3 with an Attachment Site Mutation

Mutations in the *att* region that effectively reduce site-specific recombination have been isolated (Shulman and Gottesman, 1973). The most well characterized representative of this class is *att*24, a cis-acting mutation that results in severely reduced site-specific recombination in the presence of all known phage and host integration functions. The *att*24 mutation genetically defined the 15 bp common core sequence that is found in the center of all primary *att* sites (Landy and Ross, 1977). A 100 fold reduction in recombination is seen if one or both *att* sites of λ*att*L-*att*R carry the *att*24 mutation (Shulman and Gottesman, 1973).

The nature of *int*-h3 integrase activity with *att*24-containing sites was assessed using two variants of the phage λ*att*L-*att*R. These experiments are similar to those outlined in Table 2. One variant carries the *att*24 mutation in *att*L and the other in *att*R. When either variant of λ*att*L-*att*R is grown with a phage that supplies *int*$^+$ and *xis*$^+$ gene products, the recombination frequency is 1%, in contrast to a 70% recombination frequency with normal *att* sites. When the *int*-h3 gene product is supplied, however, the recombination frequency increases to 17% for λ*att*L24-*att*R and to 11% for λ*att*L-*att*R24.

Thus Int-h3 is capable of catalyzing recombination between *att* sites with mutant core sequences. This Int-h3-catalyzed recombination requires HimA activity, however, since breakdown of the *att*24-carrying phage will not occur in the *him*A42 host even if Int-h3 is supplied (data not shown).

Discussion

In this study we report the isolation of a mutation, *int*-h3, that results in the expression of a λ integrase exhibiting enhanced activity. This activity is manifested by the ability to support λ site-specific recombination under two conditions where wild-type integrase exhibits low activity: in bacterial mutants that fail to support λ site-specific recombination, and under

conditions where the recombinational *att* sites are altered.

This enhanced integrase activity could result from three different alterations: first, a change in regulation resulting in increased synthesis of a normal integrase; second, synthesis of a more stable integrase; or third, synthesis of a more reactive integrase. The experimental evidence leads us to favor the third explanation. Arguing against explanation one is the mapping data that locates the *int*-h3 mutation within the structural gene, removed from all regions known to be involved in *int* gene expression (Shimada and Campbell, 1974). Moreover, measurement of Int protein synthesis in infected bacteria shows that λ*int*-h3 does not synthesize Int at a substantially different rate than λ*int*$^+$ (H. Miller, unpublished experiments). Arguing against explanation two is the fact that the wild-type Int is a relatively stable protein (Weisberg and Gottesman, 1971). Thus by exclusion we favor explanation three, that *int*-h3 activity results from a more reactive integrase.

Range of Activity

Although the *int*-h3 mutation was selected for its ability to catalyze site-specific recombination in the *him*A42 mutant host, the altered integrase supports site-specific recombination in bacteria carrying a variety of *him* mutations. These mutations include missense as well as nonsense mutations mapping in at least three different genetic loci. Activity in such a range of *him* mutants demonstrates that the enhanced activity of the *int*-h3 mutant protein is not allele-specific, thus ruling out the possibility that the mutant integrase suppresses a specific alteration in a particular host protein. We therefore conclude that the *int*-h3 integrase is active under conditions where at least some host components are present in low levels, or in a relatively inactive form.

The fact that λ*int*-h3 requires normal levels of HimA activity for lysogeny in *att*-deleted strains suggests that Int-h3 is not a Him-independent integrase. Confirming this assumption are recent studies with mutants that on initial characterization appear to carry deletions of the *him*A gene (H. Miller, unpublished experiments). Int-h3 is inactive in these putative deletion strains. This suggests that the missense and nonsense *him*A mutants that we have studied still retain the ability to express some HimA activity.

Activity with Nonparadigm *att* Sites

Considering that the *int*-h3 mutation was selected for the ability to support integration and excision in the *him*A42 mutant strain, it was surprising to find that the mutant integrase works effectively with *att* sites that vary from the paradigm *att* sequence. Two examples of Int action at variant sites were studied: integration at secondary bacterial *att* sites; and intermolecular *int*-promoted recombination by phages with mutationally altered *att* sites. One possible explanation for this apparent broad range of activity lies in the screening procedure used in obtaining the *int*-h3 mutation. Since the screening required that the *int*-h3 mutation result in the ability to excise a cryptic λ prophage from one secondary *att* site in the *gal*T gene in a *him*A42 background, it it possible that we biased the procedure to yield a mutation with dual characteristics. This explanation seems improbable, however, for the following reason. If excision of the cryptic prophage is a separable characteristic from the ability to function in the *him*A$^-$ background, then we would expect to find mutants capable of excising the cryptic prophage independent of the cycling procedure which selected for function in *him*A mutants. Following the cycling procedure, mutants capable of excsing the cryptic prophage in a *him*A$^-$ background were found at a frequency of 2×10^{-3}, while we were unable to directly obtain such mutants from the same initial mutagenized stock of λ at a frequency of $<10^{-5}$. The characteristic of activity at secondary *att* sites was therefore only found when the enrichment procedure requiring integration and excision at the primary *att* site in the *him*A42 background was used.

Based on this evidence, we conclude that the selection for efficient integration and excision in the *him*A host yielded the *int*-h3 mutation, which alters the Int product so that it effectively recognizes secondary or altered *att* sites. It is therefore probable that this second activity is directly associated with the ability of *int*-h3 to bypass the *him*A requirement for site-specific recombination.

Such an association of these two properties is implied by the synergistic effect on the efficiency of lysogeny of λ*int*-h3 in the presence of the combination of *him*A$^-$ and an *att*B deletion. The frequency of lysogeny in this case is several orders of magnitude less than that calculated for the product of the lysogeny frequencies observed in the cases of the single mutants. It should be noted that we might expect to observe a similar synergistic effect with wild-type Int if we could measure lysogeny frequencies as low as 10^{-10}. These studies demonstrate that lysogeny by λ*int*-h3, which reflects the efficiency of integration, shows a much greater dependence on Him factors for nonparadigm *att* sites.

The dual properties seen in λ*int*-h3 of site-specific recombination with paradigm *att* sites independent of normal HimA function, and increased activity for nonparadigm *att* sites with a greater requirement for HimA function, suggest a possible role for HimA function in *att* site recognition. Genetic and sequence analysis point to the "common" core as the essential region for *att* recognition. Site mutants defective in Int-promoted recombination map in the common core (Shulman and Gottesman, 1973), and nucleotide sequence analysis shows that secondary *att* sites consist of variations of the paradigm common core sequence

(Bidwell and Landy, 1979; Christie and Platt, 1979). Moreover, there is no other obvious similarity between secondary sites and the primary *att*B site. Thus the similarities between the secondary "core" sequences and that of the paradigm "core" probably explain preference for those secondary sites, while the differences explain the inefficiency of integration at secondary sites. If this is the case, then the fact that HimA function is required for Int-h3 activity at secondary sites and Int^+ activity at primary sites suggests that HimA function may act in the recognition of the core region. Consistent with this assertion is the finding that Int-h3 is active in recombination between *att* sites carrying the *att*24 core mutation.

Although the HimA protein may play a role in core recognition, its activity with heterologous Int systems suggest that HimA cannot be involved in the nucleotide sequence recognition specificity for the primary *att* nucleotide sequence. In the case of Int-h3, the altered integrase must recognize the "core" in the absence of normal HimA activity, but require normal activity to recognize variations of the paradigm core sequence. Perhaps HimA protein interacts with a particular structural configuration associated with the *att* site. For example, recognition of the core by integrase could be facilitated in a nonspecific manner by a protein that aids in the melting of the hydrogen-bonded duplex DNA molecule. Such a nonspecific mode of action could explain the role of HimA protein in other apparently unrelated processes such as transcription that ultimately require protein-DNA interaction.

Evolution of λ Integrase

The isolation of a mutant integrase with enhanced activity raises the question of why wild-type integrase evolved to a form having less than maximum activity. We suggest several reasons why maximum Int activity may not be desirable for either the phage or the host bacterium:

—Int synthesis is constitutive to a low level (Shimada and Campbell, 1974; Echols, 1975). A low level of a hyperactive Int could lead to prophage excision, particularly if the Int were somewhat Xis-independent (as Int-h3 appears to be). This would lead to an instability in the maintenance of the prophage.

—The level of activity of wild-type Int assures integration at *att*B. Integration at sites other than *att*B (particularly in structural genes) could be disadvantageous to the lysogen.

—Int generates deletions of λ DNA (Parkinson and Huskey, 1971). A hyperactive Int might generate deletions of required λ genes—indeed, of the *int* gene itself—at high frequencies. In this respect, the fact that the original isolate of λ*int*-h3 carried what appears to be a b_2 type of deletion is consistent with this explanation.

—Since Int probably has a nucleolytic activity (Kikuchi and Nash, 1979), an extremely active nuclease might damage DNA of the phage or the host.

—A hyperactive Int such as Int-h3 bypasses the requirement for Him proteins for integration. We have suggested that the *him*A gene coordinates the two processes required for lysogeny by providing a protein necessary for the integration reaction, and by regulating repression (Miller and Friedman, 1980). Thus a phage with a hyperactive, *him*A-independent Int might not be subject to the precise coordination of the processes necessary for choosing the lytic or lysogenic pathways.

Laboratory Uses of λ*int*-h3

We would like to point out an extremely useful aspect of the *int*-h3 mutation. Substitution of λ*int*-h3 for λ in the common technique for generating λ specialized transducing phage (Schrenck and Weisberg, 1975) should markedly simplify and improve the efficiency and sensitivity of this process, since this method is based on the integration of λ into secondary bacterial *att* sites adjacent to genes of interest. It is obvious that this process will be substantially aided by the high frequency of secondary site integration by λ*int*-h3.

Experimental Procedures

All of the following are presented in the accompanying paper (Miller and Friedman, 1980): media, lysogen formation, tests for lysogeny, and red plaque assay.

Phage Crosses

Phage to be crossed were mixed with bacteria in LBMM at a cell density of 2×10^6/ml and at an moi of 5 of each phage. The infected cells were pelleted and resuspended in TMG at one tenth volume. They were then exposed to 300 erg/mm^2 ultraviolet radiation in a glass petri dish, resuspended in the original broth, and grown to lysis. When Int-promoted recombination was studied, the phage were mixed with the bacteria and grown to lysis.

Bacteria and Phage

In addition to those cited in the accompanying paper, the following bacteria and phage listed with their relevant genotype and source were used. Phage: λcI857*att*L24-*att*R, λ*imm*434 *c int*6 *h* and λcI857*att*L-*att*R24, from M. Gottesman; λ*b*2043 and λ*bio*2037 from R. Weisberg. Bacteria: K548- carries an IS2 element in the leader region of the *gal* operon, K545-GalE$^-$, from S. Adhya; K985-*att*B deleted, from M. Gottesman; K176-Cr63, which is resistant to λ*h*$^+$ but sensitive to λ*h* mutants, from M. Yarmolinsky; K770-*hip*157, and K1039-*hip*157 red plaque strain from A. Kikuchi and R. Weisberg; K936-K37 *him*A42, K397-K387(φ80 *su*III$^+$), K734-K387 (φ80), K748-K713 (φ80), K889-K176 (λ*imm*434*h*), K1020-K985*him*A42, all from this laboratory.

Selection for λ*int*-h

Selective pressure to enrich for *int*-h phage was applied by cycling a mutagenized stock of λcI857 through alternate steps of lysogeny and induction in the *him*A42 host, K634. Mutagenesis was applied by growing the phage in the *mut*D-5 mutator strain, K481 (Fowler, Degnen and Cox, 1974). Lysogens of the *him*A host were obtained by infecting bacteria at an moi of 5 and spreading ~10^7 infected cells on LB plates seeded with 10^9 λcIh80 + 10^9λcI. Stable lysogens are immune to infection by counter-selecting phage, and thus bacteria growing on the plate will be either lysogens or mutants resistant to λ infection. Plates spread with infected bacteria were incubated over-

night at 32°C and were found to contain approximately 500 colonies. The bacteria from five plates were collected, washed with TMG, and resuspended in LB broth.

The pooled bacteria were treated with a heat pulse in the following manner to induce any lysogens. Bacteria resuspended in LB broth were grown at 34°C for 2 hr. Cultures were shifted to 42°C, a temperature at which the cI857 repressor denatures (Sussman and Jacob, 1962), in order to release any lysogens from repression. After 30 min of growth at 42°C, cultures were shifted to 37°C to permit completion of phage production at this temperature, which is more optimal for phage growth. The resulting lysate was treated with chloroform to kill bacteria and clarified by low-speed centrifugation.

Starting with this preparation of phage, the lysogeny and induction procedure was repeated through two more cycles.

Phage lysates produced by the cycling procedure were screened for excision activity using a *him*A derivative (K713) of the red plaque strain (Enquist and Weisberg, 1976). Details of the red plaque test are outlined in the accompanying paper (Miller and Friedman, 1980). When the *him*$^+$ red plaque strain is used as a bacterial lawn, the formation of red plaques indicates Int and Xis activity. When the *him*A42 red plaque strain, K713, is used in a similar manner, an *int*$^+$ *xis*$^+$ phage forms colorless plaques.

Based on the reasonable assumption that this failure to form red plaques is due to the restrictive effect of the *him*A mutation, we screened a lysate obtained by the cycling procedure for phage capable of forming red plaques on a lawn of K713 at 32°C. Red plaques were observed at a frequency of 2×10^{-3}. Red plaques could not be found in the mutagenized lysate prior to cycling ($<10^{-5}$). The ability to form a red plaque on the *him*A$^-$ red plaque strain is used as the principle means of identifying the Int-h3 phenotype.

Test for Integration at *att*B

Integration at the normal *att*B site was determined using a test that is based on the fact that *att*B is located close to the *gal* operon. Transcription from the P_L promoter of a λ prophage integrated at *att*B can proceed into the *gal* operon with the net result of a substantial increase of *gal* expression (Yarmolinsky, 1963). This λ-promoted expression occurs even when the *gal* regulatory region carries an IS2 element (for example, *gal*308), because transcription complexes modified by the λ N protein can transcend the transcription barrier imposed by IS2. Prophages inserted at sites distal from the *gal* operon do not show this effect. Thus increased production of *gal* enzymes following induction of a λ prophage indicates that integration is probable at the *att*B site. We feel confident in making this assumption, since no secondary *att* sites that are sufficiently close and upstream from the *gal* operon have been identified (Shimada et al., 1972).

To test for integration at *att*B, lysogens of a thermoinducible (cI857) phage are constructed in K548, a strain that carries the *gal*308 insertion (in the leader region of the *gal* operon) and is therefore Gal$^-$. The lysogens are transferred to MacConkey galactose indicator plates and incubated overnight at 32°C. The plates are then shifted to 41°C. After 3 hr, a color shift from colorless to red indicates *gal* operon expression initiating from P_L. The efficacy of this test is demonstrated by the observation that a λ prophage integrated at a distal site, the *att* site for φ80, does not exhibit such Gal expression following thermoinduction.

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