Site-specific Recombination of Bacteriophage λ: The Role of Host Gene Products

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Integration of the DNA of bacteriophage λ into the chromosome of *Escherichia coli* occurs by recombination at two unique genetic loci: *attP* on the phage genome and *attB* on the bacterial chromosome. This reaction is therefore an example of site-specific recombination. The only phage-encoded protein required for recombination at these *att* (attachment) sites is Int, the product of the phage *int* gene. Integration of λ generates a prophage that is flanked by two new *att* sites, *attL* and *attR*. Excision, recombination between *attL* and *attR*, requires Xis, the product of the phage *xis* gene, as well as Int (for review, see Weisberg et al. 1977).

It has previously been demonstrated that in addition to phage-encoded proteins, the products of one or more host genes are necessary for λ site-specific recombination (Miller and Friedman 1977a; Nash et al. 1977; Williams et al. 1977). In this paper, we briefly present the results of experiments that characterize mutant *E. coli* strains that are unable to carry out λ site-specific recombination. Our studies describe the biochemical nature of the integration defect in these strains and define the genetic loci that are responsible. The interest in these newly identified genetic loci promises to extend beyond the study of λ site-specific recombination, since the mutants exhibit various other phenotypes, some of which do not appear to be a consequence of a defect in recombination.

RESULTS

Selections for Host Mutants Deficient in λ Integration

Two different selection schemes were used for isolating mutants defective in λ integration. The basis for each selection is illustrated by the appropriate diagram and briefly described below. Enrichment for mutants by mutagenesis was employed in both selections.

Selection A. This selection (by H.I.M. and D.I.F.) employs a λ variant—λ*N*⁻*int-c cam* (see Fig. 1). Integration of this phage variant into the *E. coli* chromosome results in cell death. Thus, mutant cells unable to integrate λ will survive (for a detailed explanation, see Miller and Friedman 1977a). Mutant cells obtained by this selection contain mutations that are designated *him* for host integration mediators. Several mutations obtained by this procedure were mapped to a single locus (*himA*). The selection was then repeated in a strain diploid for this region. An additional strain, which was shown to contain at least two mutations (*himB114* and *himC*), was obtained in this manner (see Genetic Mapping).

Selection B. This selection (by A. K. and R. A. W.) is illustrated in Figure 2. The procedure selects for the maintenance of bacterial genes, the *gal* operon, that are flanked by *attP* and *attB*, the substrates for Int-promoted recombination. In the presence of Int, only mutant cells unable to delete the bacterial DNA can form *gal*⁺ colonies on differential media. The mutation obtained using this procedure is referred to as *hip*, for host integration protein.

Characteristics of Mutant Strains

Several easily scored phenotypes that were common to all or many of the mutant strains were used to verify the presence of the mutations in mapping and other experiments. These are discussed below.

Lysogeny assay. This assay tests the ability of a strain to form immune lysogens using the EMBO plate method of Gottesman and Yarmolinsky (1968). Both phage integration and the establishment of prophage immunity are required for a positive lysogeny test. Strains carrying the *himA*, *himC*, and *hip* mutations fail to form immune lysogens by this test. The failure of λ to integrate in these strains is the most likely explanation for the negative lysogeny test, since establishment of repression appears to be normal as judged by the ability of λ to form turbid plaques on these strains. Similar results were obtained for lysogeny tests using φ80 and P2; temperate phages whose *att* site specificities differ from that of λ.

Excision of λ from a secondary att site. Prophage λ has been isolated at sites other than *attB*. One such site is found within the *galT* gene. The prophage inactivates the *galT* gene, but prophage excision,

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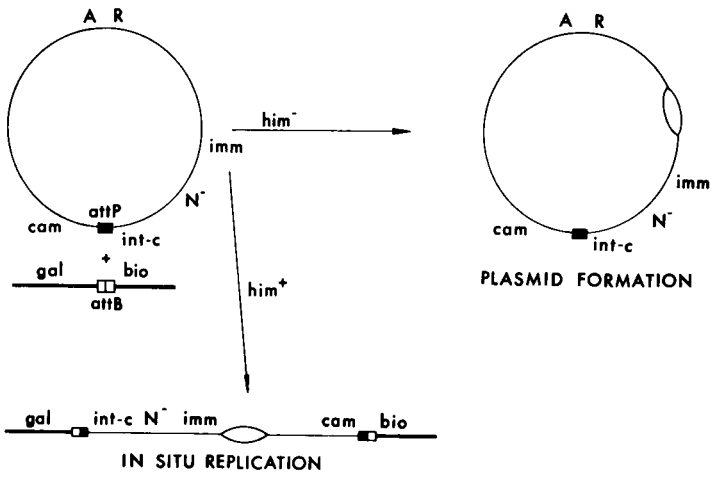

Figure 1. Selection for host integration mutants (A). Phage λ*N* carrying the *int-c* (integrase constitutive) mutation (Shimada and Campbell 1974) is capable of inserting into the chromosome of an infected bacterium at high frequency, (*him*$^+$ condition). Once inserted, the prophage can replicate in situ because repression cannot be established in the absence of the *N*-gene product. The in situ replication results in the death of the bacterial host. However, if the bacterium is incapable of supporting the insertion of the phage (the *him*$^-$ condition), the phage replicates in the form of a plasmid, a situation not lethal to the bacterium. The presence of a determinant for chloramphenicol resistance on the phage chromosome (*cam*) and the presence of chloramphenicol in the selective media eliminates the recovery of uninfected cells. The bubbles on the phage chromosome represent Cairns-type replication forks. For more details, see Miller and Friedman (1977a).

which is Int- and Xis-dependent, restores a functional *galT* gene (Shimada et al. 1972). This reaction is conveniently tested by the red-plaque assay developed by Enquist and Weisberg (1976). Strains with mutations *himA, himB, himC,* or *hip,* carrying prophage λ in the *galT* gene, all fail to excise the prophage when Int and Xis are supplied by infection with λ.

Growth of bacteriophage Mu. Bacteriophage Mu is unable to form plaques on *himA*, *himB* or *hip* strains. Phage growth is severely reduced even following induction of lysogens containing thermoinducible Mu *cts* prophages (Miller and Friedman 1977b). Mu gene expression appears to be greatly affected, since *himA* and *hip* strains survive killing by Mu either following infection or induction of Mu *cts* lysogens (Miller and Friedman 1977b; Miller 1977; Giphart-Gassler et al., this volume). We have not yet determined if the nature of the block in Mu development in these strains is caused by a block in site-specific recombination.

Growth of λ terminator variants. Certain variants of λ that have mutations in the λt_{R1} transcription terminator, λ*cin* (Wulff 1976; Rosenberg et al. 1978), are unable to form plaques on *himA* or *hip* mutant strains. No obvious relationship of these phenomena to site-specific recombination suggests itself. Therefore, the failure of λ*cin* to grow on these mutant strains may be due to functions of the *hip* and *himA* genes independent of their role in recombination.

The failure to support the growth of λ*cin* was the basis for the isolation of another mutant strain, *hid*, which was subsequently found to be defective for λ site-specific recombination (Williams et al. 1977). The *hid* mutation maps in the *himA* gene (see below). However, strains carrying the *hid* mutation differ from *himA* mutant strains in that they are permissive for the growth of Mu. This is apparently due to the leaky phenotype of the *hid* mutation.

The various characteristics of the mutant strains described above are most likely the result of single genetic alterations. In mapping experiments, all of the characteristics segregate together. Moreover, for one

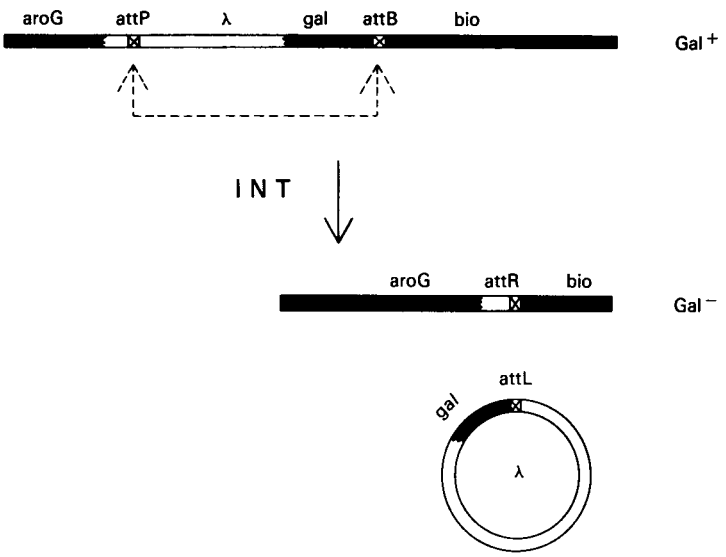

Figure 2. Selection for host integration mutants (B). A lysogen of λ*gal* whose prophage is bracketed by the two attachment sites active for integrative recombination, *attP* and *attB*, is infected at a high moi by λ*int-ch*80 and λ*int-chλ* and plated on EMB-galactose plates. The *int-c* mutation insures high-level production of Int, although the infecting phage is repressed by the lysogen. The *h*80 and *hλ* (φ80, λ adsorption specificities) minimizes the number of phage-resistant cells. The excised prophage, which is repressed, does not replicate and is lost with cell division. Prophage excision, which in this lysogen is dependent on Int and host proteins alone, deletes the only functional *gal* operon in the cell. Thus, cells unable to perform prophage excision should maintain a functional *gal* operon and form *gal*$^+$ colonies on EMB-*gal* plates. This figure indicates the fate of a normal cell capable of excising the prophage. Colonies surviving this selection were screened by assaying recombination of λ*attB-attP* to verify the integration-negative phenotype (see below).

mutation, the *himAam79* mutation, all of the mutant phenotypes can be eliminated by the introduction of a *su*III$^+$ amber suppressor (Miller 1977).

Genetic Mapping

Figure 3 shows the map locations of the *himA*, *himB*, and *hip* mutations on the *E. coli* linkage map. Map locations were determined by a combination of Hfr mating, F′ episome complementation, and P1 cotransductional mapping.

The *hip* mutation maps near 19 minutes and is cotransducible with *aroA* (20%) and *serS* (50%). Introduction of an episome containing this region (F′147) complements the *hip* phenotype indicating that the *hip* mutation is recessive to wild type.

The *himA* mutations map near 38 minutes immediately adjacent to the *pheS* and *pheT* genes (95% cotransducible) as does the *hid* mutation. All of these mutations are complemented when wild-type genes are introduced either by an episome (F′148) or by several specialized transducing λ phages carrying the *pheS* region (Miller 1977). In addition, complementation experiments with pairs of mutant alleles using the transducing phage carrying one of the mutations indicated that they all occur in the same gene.

As mentioned above, a strain was isolated that was shown to contain at least two mutations, *himB114* and *him*C. This is inferred from the fact that transductants that cross out the *himB114* mutation, assayed by the ability to propagate Mu, are still highly defective for λ integration. Indeed, these transductants are considerably more defective for λ integration than the original mutant strain. Thus, the *himB114* mutation partially suppresses the defect for λ integration in the *himC* strain, even though the *himB* mutation alone blocks excision of λ from a secondary *att* site (see above). The mutation conferring the *himC* phenotype has not been mapped.

The *himB* mutation, scored as failure to plate Mu, maps at 82 minutes and is 98% cotransducible with *dnaA* and more than 99.5% cotransducible with *cou*^{r}, a mutation in a subunit of DNA gyrase that confers resistance to the antibiotic coumermycin (Gellert et al. 1976). The mapping data, together with experiments measuring DNA gyrase activity in *himB*$^-$ strains, suggest that this mutation may map in a gene coding for one of the subunits of DNA gyrase (M. Gellert, pers. comm.).

Generalized Recombination

Generalized recombination was measured in the mutant strains by several methods: (1) the frequency of recombination in Hfr × F$^-$ crosses, (2) the frequency of P1-mediated transduction, and (3) crosses between *red* phage λ using genetic markers on the same side of the attachment site. By all methods homologous recombination in the mutant strains was not grossly different from that in the wild-type strains.

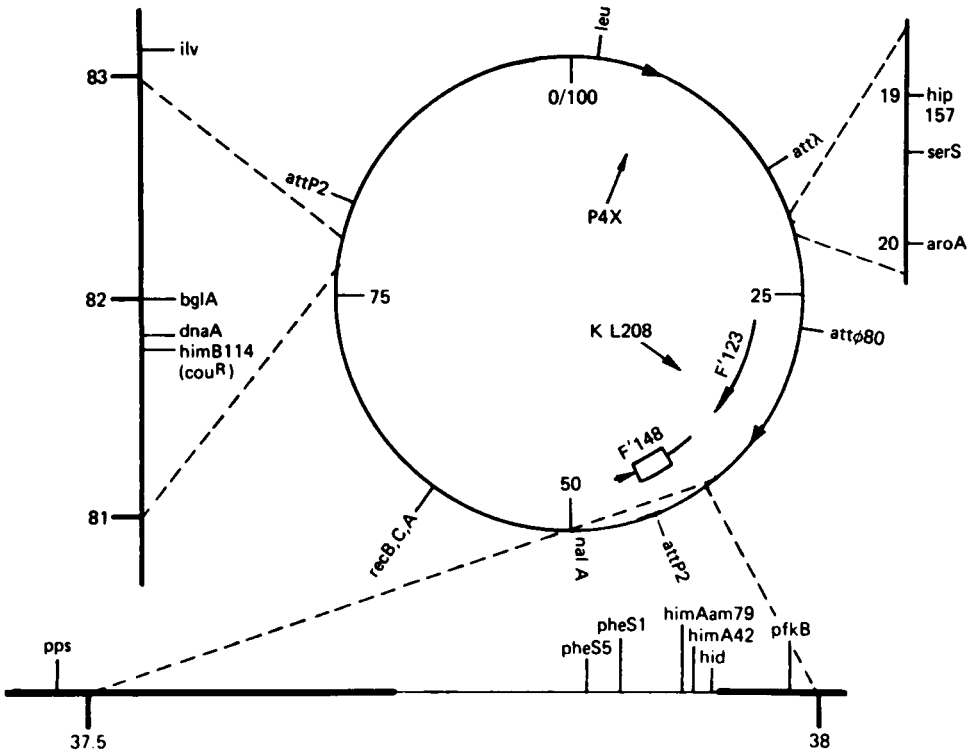

Figure 3. Map locations of host integration mutants. Simplified linkage map of *E. coli* showing the position of genetic loci described in the text as well as the position of some F′ episomes and the origins of Hfr strains. The thin line in the expanded *himA* region delineates the region of homology with a λ*pheS* transducing phage. The orientation of this region may be the reverse of that shown (Miller 1977; Springer et al. 1977).

Quantitative Studies on Site-specific Recombination

Integrative recombination of λ was measured by determining the frequency of formation of immune lysogens and loss of bacterial genes during lytic growth of λ*attB-attP* (Nash 1975). The results shown in Table 1 indicate that lysogen formation in *himA*, *hip*, and *himC* strains is reduced by several orders of magnitude as compared with wild-type controls. This result parallels the reduction in recombination of λ*attB-attP* in the *himA* and *hip* strains. However, recombination of λ*attB-attP* in the *himC* strains appears normal. It should be pointed out that Int-promoted recombination during lytic crosses between λ*attB* and λ is greatly reduced in *himC* strains (data not shown). Excisive recombination, measured by loss of bacterial genes during lytic growth of λ*attL-attR*, is also reduced in *himA* strains, but not to the extent of integrative recombination. Excisive recombination in *hip* strains is also more efficient than integrative recombination (Enquist et al., this volume).

Except for excision of λ at the secondary *galT att* site, we can find no defects in the *himB* mutant strain for integrative or excisive recombination (data not shown).

A λ *int*-gene Mutant That Bypasses the Requirement for Host Integration Gene Function

We have selected for mutants of λ that promote excision of a *galT* λ prophage of a *himA*$^-$ strain (red-plaque assay; see above) (Miller 1977; Miller and Friedman 1977b). These phage mutants also give positive red-plaque tests with *himB-*, *himC-*, and *hip*-containing strains. One mutation, called *int-h*3 (integrates in *him*), has been mapped within the λ *int* gene. Both λ lysogeny and integrative recombination of λ*attB-attP* are catalyzed efficiently under *himA*$^-$ or *hip*$^-$ conditions when Int is supplied by λ*int-h*3 (data not shown). The Int-*h* phenotype is constitutive in *int-c int-h* lysogens.

One additional characteristic of particular interest is the ability of phages carrying the *int-h* mutation to integrate into *attB*-deleted hosts. In contrast to the 200-fold reduction in lysogeny frequency shown by λ under these conditions (Shimada et al. 1972), λ*int-h* shows only a two- to threefold reduction. Thus, the *int-h* mutation appears to render both the bacterial *att* site and host integration genes dispensable for λ integration.

Table 2. Production of Int by Mutant Lysogens

Source of Int	Amount of extract (μg protein)	Recombination (%)
—	—	<1
N99 (λ*int-c*)	0.7	<1
	2.1	27
	6.2	58
N99*himA42* (λ*int-c*)	0.7	2
	2.1	27
	6.4	43
KL229*hip157* (λ*int-c*)	0.8	7
	2.3	28
	7.0	32

In each recombination reaction, 0.15 μg tritium-labeled supertwisted λ*attB-attP* DNA is added to a 64-μl mixture containing 39.1 mM Tris-HCl (pH 7.4), 39.1 mM KCl, 11.7 mM spermidine, and 7.8 mM sodium EDTA. After 10 min at 25°C, 500 μg bovine serum albumin (BSA), 125 μg *E. coli* host integration factor, and the indicated amount of Int preparation are added in 50 μl of 50 mM Tris-HCl (pH 7.4). The reaction mixture is incubated for 15 min at 25°C and then analyzed for recombined DNA by the filter method described in Nash et al. (1977) and Kikuchi and Nash (1978). The *E. coli* host integration factor is prepared as described in Mizuuchi and Nash (1976) and consists of a dialyzed high-speed supernatant of a crude extract of a *recB21* derivative of strain N99. The Int preparations are dialyzed crude extracts of cells carrying a single λ*int-c226* prophage. These strains are lysogens of wild-type strain N99, strain N99*himA42*, and strain KL229*hip157*, as indicated. Growth, collection, and extraction of cells is as described in Nash (1975).

In Vitro Characterization of Host Mutants

Integrative recombination occurs in cell-free extracts (Nash 1975). This reaction requires a supertwisted substrate DNA (Mizuuchi et al. 1978) and extracts that contain both the phage *int*-gene product and host-encoded factors (Kikuchi and Nash, this volume). We have used the in vitro reaction to investigate the basis of the in vivo defect in integrative recombination of *himA* and *hip* strains. Table 2 shows that *int-c* lysogens of both kinds of mutant cells produce active Int in essentially normal amounts. This makes it unlikely that altered viral gene expression is the cause of defective recombination in these mutants. On the other hand, the data of Table 3 (lines 1, 2, 6, and 7) show that host factors isolated from mutant

Table 1. Integrative and Excisive Recombinations

Bacteria	Allele	Recombination (%)		
		lysogeny[a]	λ*attB-attP*[b]	λ*attL-attR*[b]
K-37		90	34	68
K-648	*himAam79*	<0.1	0.2	3
K-634	*himA42*	<0.01	0.1	2
K-1025	*himC*	0.1	24	n.t.
NK-5	+	95	46	n.t.
NK-6	*hip157*	<0.01	0.6	n.t.

The first four strains are isogenic, as are the last two. Except for K-1025, all mutations were introduced by P1 transduction. n.t.=not tested.

[a]Experimental procedures are described in Miller (1977).

[b]Procedure described in Miller and Friedman (1977a).

Table 3. Complementation between Extracts of Mutant Cells for In Vitro Integrative Recombination

Amount (μg protein) and source of extract				
himA	*himA* (λ*int-c*)	*hip*	*hip* (λ*int-c*)	Recombination (%)
—	—	145	14	<3
—	—	290	14	<1
14	—	145	14	13
47	—	145	14	20
142	—	145	14	24
108	19	—	—	<1
285	19	—	—	<1
108	19	2	—	18
108	19	6	—	35
108	19	17	—	38
108	19	172	—	37

Reaction mixtures are as described in the legend to Table 2, except that *E. coli* host integration factors are derived from cells of strain N99*recB21himA42* or KL229*hip157* instead of from cells of strain N99*recB21*.

cells fail to supplement active Int for integrative recombination in vitro. For the *himA* strain, this is consistent with an earlier study with the *hid* mutation, which yielded similar results (Williams et al. 1977). It should be pointed out that in the present study the substrate is provided in the supertwisted form. Dye–bouyant-density analysis of DNA from mutant or wild-type reaction mixtures shows that a majority of the DNA remains supertwisted (data not shown). Thus, the failure to recombine does not simply represent the loss of supertwisted substrate in the presence of the mutant extracts. Nor do the mutant cells produce other kinds of diffusible inhibitors. Mixtures of wild-type and mutant extracts carry out recombination efficiently (data not shown). Furthermore, as shown in Table 3 (lines 3–5, 8–11), the two mutant extracts complement each other in vitro. Thus, both the *himA* and *hip* mutants are missing a different factor or set of factors required for integrative recombination. The experiments described in Table 3 define an assay for these missing factors and their purification is in progress.

DISCUSSION

We have presented evidence that demonstrates the existence of several genetic loci in *E. coli* that are involved in site-specific recombination. For the two most extensively characterized loci, *himA* and *hip*, we have shown that cell-free extracts from these strains lack different factors necessary for catalyzing in vitro λ integrative recombination. Further work will be needed to decide if the in vitro activities we attribute to the *hip*- and *himA*-gene products are indeed encoded by the two genes or reflect instead the action of proteins under their control. In either case, the strikingly similar phenotypes of the two mutant strains suggest that the two gene products or the proteins under their control participate together in their functions or act sequentially in a common pathway.

Perhaps the most intriguing aspect of these mutants is their pleiotropy. Several effects apparently unrelated to recombination have been noted, but this is by no means an exhaustive list. Experiments indicate an involvement of these genes in genetic transposition, plasmid stability, and chromosomal duplication. It would be simple to reconcile the pleiotropy of these mutations if the gene products acted indirectly at the level of regulation of gene expression. However, if the different effects all resulted from a direct loss of the gene product activity, a general role in DNA-protein interaction could account for the various characteristics.

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