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Deletion, Recombination and Gene Expression Involving the Bacteriophage λ Attachment Site

KEVIN STRUHL†

Medical Research Council Laboratory of Molecular Biology
Hills Road, Cambridge, CB2 2QH, England

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I analyse illegitimate recombination events mediated by the bacteriophage λ *int* gene by comparing the DNA sequences of a bacteriophage λ hybrid containing the *Saccharomyces cerevisiae* (yeast) *his3* gene and five independently derived deletion mutants. Behaviours of these λ*his3* deletion mutants with respect to *int*-mediated recombination and to *his3* gene expression are examined.

Observations made are the following.

(1) Deletion break points in the yeast DNA occur only at sites that have partial sequence homology to the core of the attachment site.

(2) Deletion break points at the λ attachment site occur at two or more distinct locations within the common core, thus generating new hybrid core sequences.

(3) In two instances, the recombination events generate unusual sequences at the crossover point.

(4) Three deletions represent alternative outcomes of recombination with the same yeast DNA sequence.

(5) Phages differing only in hybrid core sequences behave quite differently in *int*-mediated recombination and express *his3* at different levels.

Conclusions suggested from these observations are as follows.

(1) *In vivo* support for the idea that *int* protein produces staggered breaks in the attachment site core.

(2) Sequences to the left of the core influence *int*-mediated recombination.

(3) Deletions are formed by a break and join mechanism rather than by strand displacement.

(4) The hybrid core regions can provide a Pribnow box that, when fused to appropriate sequences, forms a leftward promoter for *his3* expression.

(5) *int*-mediated recombination produces a diversity of sequences at the novel joints that have different functional consequences.

1. Introduction

Site-specific recombination occurs when a special gene product interacts with a specific DNA sequence to promote an exchange of genetic material. Such a mechanism is or may be involved in the chromosomal movement of controlling elements (McClintock, 1956), temperate prophages (Campbell, 1962), insertion sequences and prokaryotic transposable elements (Bukhari *et al.*, 1977), yeast

† Present address: Department of Biological Chemistry, Harvard Medical School, 25 Shattuck St., Boston, Mass. 02115, U.S.A.

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mating type cassettes (Hicks *et al.*, 1977), somatic recombination between immunoglobulin genes (Hozumi & Tonegawa, 1976), and integration of retroviruses into the host genome (Hughes *et al.*, 1978). In many of these cases, gene expression is altered following a site-specific recombination event.

The best studied example of site-specific recombination is that mediated by the *int* protein of bacteriophage λ. The *int* gene was so named because it promotes the highly efficient and highly specific integration of the λ genome in the chromosome of the host *Escherichia coli* cell (Zissler, 1967; Gingery & Echols, 1967; Gottesman & Yarmolinsky, 1968). From a series of genetic experiments, it was suggested that the phage attachment site (POP′) shared a common core sequence with the bacterial attachment site (BOB′) (Shulman & Gottesman, 1973). Subsequent DNA sequence analysis showed a 15 nucleotide pair region (the O region or core) present in the original phage and bacterial attachment sites, and in both hybrid sites generated by the recombination event (Landy & Ross, 1977). Further analysis indicated that secondary (less frequently used) attachment sites in the *E. coli* genome have significant but imperfect homology to the common core (Bidwell & Landy, 1979; Christie & Platt, 1979). In addition to prophage integration, the *int* gene is responsible for high frequency site-specific recombination between λ phages (Guerrini, 1969) and for a class of deletion mutants of bacteriophage λ (Parkinson, 1971). The deletion events occur at low frequency and are presumably the result of illegitimate recombination associated with site-specific recombination. Sequence analysis of several deletion mutants directly confirmed the hypothesis that one of the deletion break points always occurs near, but not at, the end of the core (Hoess & Landy, 1978). Evidence that the *int* protein promotes recombination by interacting with the 15 base-pair common core sequence also comes from DNA binding studies using purified λ DNA and *int* protein (Kikuchi & Nash, 1978; Ross *et al.*, 1979).

In the course of experiments on the *his3* gene of *Saccharomyces cerevisiae* (yeast), deletion mutants of a λ*his3* hybrid phage were isolated (Struhl & Davis, 1980). Most of these deletion mutants have genetic and physical properties indistinguishable from those of classical *int*-mediated deletion mutants of λ. Because these deletion mutants create novel joints between DNA and yeast DNA, they, by definition, represent illegitimate recombination events. In this paper, I determine the nucleotide sequences at and around the joint of five *int*-mediated deletion mutants of a λ*his3* hybrid. The sequences of these deletions represent the final products of *int*-mediated recombination. Because the nucleotide sequences of the relevant *his3* region and of the λ attachment site have been determined (Landy & Ross, 1977; Struhl & Davis, 1981), it is possible to examine the crossover that produced the novel joint at the DNA sequence level. The effects of novel joints of different deletion mutants on *int*-mediated recombination and on gene expression are examined.

The results bear on the following issues.

(1) Sites for *int*-mediated deletion formation and the nature of the crossover event.

(2) The mechanism of "illegitimate" *int*-mediated recombination.

(3) The core of the λ attachment site and promoter function.

2. Materials and Methods

(a) *Phages*

The *λhis3* deletion mutants used in this study (see Fig. 1) all derive from λgt4-Sc2601 and were isolated by virtue of their resistance to treatment with chelating agents. All were selected to have an intact *his3* structural gene and 6 (λgt-Sc2639, λgt-Sc2666, λgt-Sc2667, λgt-Sc2669, λgt-Sc2670 and λgt-Sc2671) were shown to have genetic properties indistinguishable from classical *int*-mediated deletions (Struhl & Davis, 1980). The *b*522 deletion was contained in the phage λgt6-*ara*6 (Struhl & Davis, 1977). The *sex*1 derivative of λgt-Sc2671 was constructed by Struhl *et al.* (1980); λgt-Sc2639 *sex*1 was made analogously. The phages used to cross in the *b*1319 deletion (see Fig. 4) and the *bio*256 substitution were, respectively, λ1096 (h^{80} *att*80 *tonB trpD b*1319 *c*I857 *chi*3) and λ305 (h^{80} *bio*256 *c*I857); these were obtained from Sydney Brenner.

(b) *Phage crosses*

The procedures and strains used for the propagation and crossing of phages were described previously (Struhl *et al.*, 1980; Karn *et al.*, 1980). In these experiments, all crosses were done in EQ82, a strain permissive for phages with either λ or *phi*80 host range. *λhis3* derivatives containing either *b*1319 or *bio*256 were constructed by crossing the *λhis*3 phages with λ1096 or λ305, respectively. Appropriate recombinants were selected on Q359 (a P2 lysogen of C600). This strain restricts phages with the *phi*80 host range (such as the λ1096 and λ305 parents) and those containing the gamma gene of λ (such as the *λhis3* parents) (Zissler *et al.*, 1971). Because phages lacking the gamma gene grow extremely poorly, the relevant ones contain a *chi* mutation which permits better growth (Lam *et al.*, 1974; Henderson & Weil, 1975). The *bio*256 substitution contains such a site (Malone & Chattoraj, 1975). The pairs of phages used in these crosses have no homology between the selective markers except for their hybrid core regions. Thus, this scheme provides a direct selection for recombinants as rare as 10^{-8}. The strain used for testing phages for *int* was LE292 (Enquist & Weisberg, 1976).

(c) *Determining the* his3 *phenotype*

The lytic "plaque without a lawn" assay of Struhl *et al.* (1980), which depends upon readthrough transcription from P_L, was employed to determine whether *λhis3* derivatives contained the intact structural gene.

To assay *his3* expression in the absence of readthrough transcription from the major λ promoters, the phages were integrated into the chromosome of *hisB*463. It was of primary importance to determine if such "lysogenic expression" was dependent on P_{int} or on some other promoter. Because the integration properties of the phages tested depend upon their *int*, *att* and P_{int} genotype, such lysogens were produced in a variety of ways.

In method 1, this was achieved either by co-infection with a wild-type λ phage or by infection into a pre-existing wild-type λ lysogen followed by immediate selection for growth of the cells in the absence of histidine. Because the "integration helper" phage contains *int*, the *λhis3* phages enter the chromosome predominantly by *int*-mediated recombination if they contain a partially or fully functional *att* site; in the absence of *att* function, they integrate by general recombination *via* λ sequence homology. Interpretation of the results produced by this method is complicated by *int*-mediated recombination between the integration helper and the phage of interest. Such recombinants could effectively fuse P_{int} back to *his3* derivatives lacking it.

Method 2 was available only for phage containing *int* and at least a partially functional *att* site. Phage were spotted on *hisB*463 and cells from the middle of the plaque were streaked for single colonies. As these experiments all involved phage containing the *c*I857 mutation, lysogens were identified by their inability to grow at 42°C and for their ability to release phage at this temperature. Because all the relevant phages tested by this method contain the ΔOP′ attachment site, their frequency of lysogen formation is considerably above that of phages lacking *int* and/or *att*; thus they most probably integrate at the normal bacterial

attachment site. However, because the frequency of lysogeny is considerably below that of wild-type phages even though the amount of repressor produced is almost certainly the same, it is likely that these lysogens contain one copy of the prophage genome/cell. These lysogens were then tested for their *his3* phenotype. This method could not be used for phages lacking *int* because the frequency of stable lysogen formation is sufficiently low to make direct selection difficult. Selection for λ-immune colonies predominantly results in abortive lysogens which are difficult to analyse for reasons of prophage segregation and cell lysis caused by prophage induction under conditions of histidine starvation produced by the complementation assay (Struhl *et al.*, 1976,1979,1980).

In method 3, phages were singly infected at high multiplicity into *hisB*463 and directly selected for growth in the absence of histidine. Int^+ phages integrate at the λ attachment site at relatively high frequency *via int*-mediated recombination. For the *bio*256 derivatives, chromosomal integration occurs at a lower frequency because it depends upon homologous recombination between the *bio* sequences. For the derivatives that cannot recombine by site-specific recombination or by general recombination (the *b*1319 and *b*522 derivatives), the frequency of integration is extremely low and His^+ complementation might not be detected (Struhl *et al.*, 1976).

Generally, His^+ complementation is not observed in the presence of 1 mM-aminotriazole, a competitive inhibitor of imidazoleglycerolphosphate dehydratase. λ*his3* phages can complement *hisB*463 under these conditions if they produce greater than normal levels of the yeast enzyme (Brennan & Struhl, 1980). Therefore, to test the approximate level of *his3* expression, lysogens were grown in the presence of 1 mM-aminotriazole, 1 mM-adenine, and 1 mg vitamin B1/ml.

(d) *DNA sequence analysis*

The deletion endpoints within yeast DNA were precisely mapped after cloning the *Bam*HI DNA fragments containing the *his3* gene and the novel joint between λ and yeast DNA into the YRp7 vector (Struhl & Davis, 1981). Cleavage of each of the resulting YRp7 hybrid DNAs with *Hin*fI endonuclease generates a fragment containing the novel joint. Because the distance between the joint at the left end of the common core and the *Hin*fI site in λ DNA is 201 base-pairs (Landy & Ross, 1977), the distances between the *Hin*fI site in the *his3* DNA and the joints may be calculated. For Sc2639 and Sc2670, the relevant *Hin*fI DNA fragment is 290±3 base-pairs in length so this distance is 90 base-pairs; for Sc2666, Sc2667 and Sc2669, this fragment is 320±3 base-pairs so the distance is 120 base-pairs. From the relevant *Hin*fI site in wild-type *his3* DNA, a *Taq*I and 2 *Mbo*II cleavage sites are located, respectively, 100, 90 and 110 base-pairs away (Struhl & Davis, 1981). As expected, Sc2666, Sc2667 and Sc2669 retain these sites while Sc2639 and Sc2670 do not.

The purification scheme for DNA fragments to determine the sequence of each deletion mutant is shown in Fig. 1. From J. A. Jaehning, I obtained the relevant *Bam*HI-*Hin*dIII DNA fragments purified by velocity sedimentation in sucrose. DNAs from Sc2666, Sc2667 and Sc2669 were cleaved with *Taq*I endonuclease, while DNAs from Sc2639 and Sc2670 were cleaved with *Hin*fI endonuclease. The resulting mixtures of DNA fragments were treated first with calf intestinal alkaline phosphatase to remove the 5′-terminal phosphates and then treated with T4 polynucleotide kinase and ATP labelled in the gamma position in order to complete the phosphate exchange. Finally, the labelled fragments were cleaved at a site within λ DNA with *Ava*I endonuclease and electrophoretically separated in a 5% (w/v) polyacrylamide gel. In each case, the fragment containing the novel joint, now labelled only at one end, was purified. The sequences of these fragments were determined by the chemical, base-specific, partial cleavage method of Maxam & Gilbert (1977).

3. Results

(a) *Structures and DNA sequences of five* λhis3 *deletion mutants*

The structures of the initial λ*his3* hybrid phage λgt4-Sc2601 and of five deletion mutants derived from it are shown in Figure 1. All five deletions were selected to

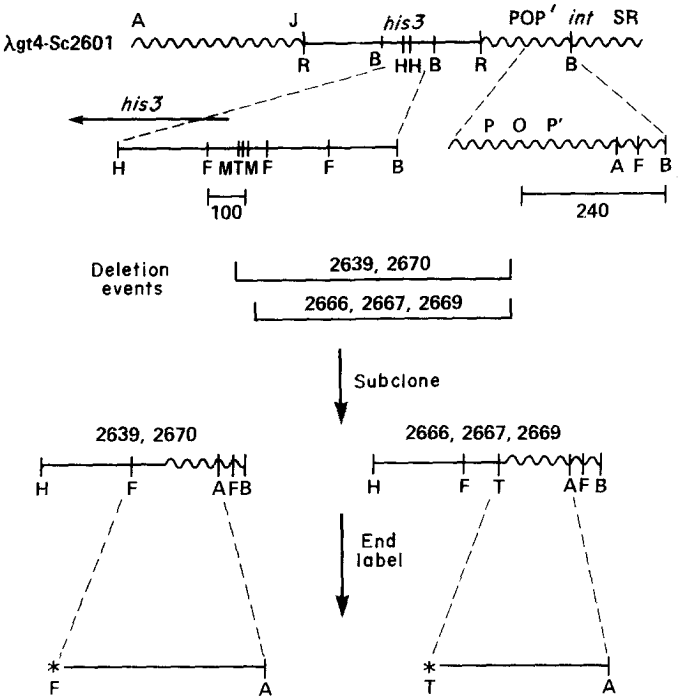

FIG. 1. Determining the DNA sequences of λ*his3* deletion mutants. The top line indicates the structure of λgt4-Sc2601 DNA. λ DNA sequences are indicated by wavy lines; yeast sequences by solid lines. Genes are shown above the line. Some restriction endonuclease cleavage sites are indicated by vertical lines (R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII). The second line expands both the *his3* and the λ*att* region. The location of the 5'-end and direction of transcription of the *his3* gene is shown. The distance from the common core of the λ attachment site (O region) to the nearest *Bam*HI cleavage site is indicated. Other restriction endonuclease cleavage sites (F, *Hin*fI; M, *Mbo*II; T, *Taq*I; A, *Ava*I) are drawn to scale. The approximate mapping positions of 5 deletion mutants are diagrammed. *Bam*HI-*Hin*dIII-generated DNA fragments containing the novel joint were subcloned into pBR322 DNA (J. A. Jaehning, unpublished results). Such DNA fragments (structures indicated on the second line from bottom) were end-labelled as described in Materials and Methods. The asterisk indicates the position of the labelled end.

have an intact *his3* structural gene and to fuse yeast DNA sequences to the common core of the λ attachment site (Struhl & Davis, 1980); therefore, they represent the outcomes of classical, illegitimate, *int*-mediated, recombination events.

Previously, the deletion endpoints within the yeast DNA had been mapped roughly. They were precisely mapped after subcloning the relevant DNA fragments into the vector YRp7, and their DNA sequences at and around the deletion endpoint determined (see Materials and Methods). The results obtained from this sequence analysis are presented in Figure 2. The deletions are viewed as the outcomes of recombination events between the attachment site and yeast DNA. Accordingly, the DNA sequences at the novel joints are compared to the sequences of the common core of the attachment site (Landy & Ross, 1977) and the wild-type *his3* sequences immediately adjacent to the joints (Struhl & Davis, 1981). Each

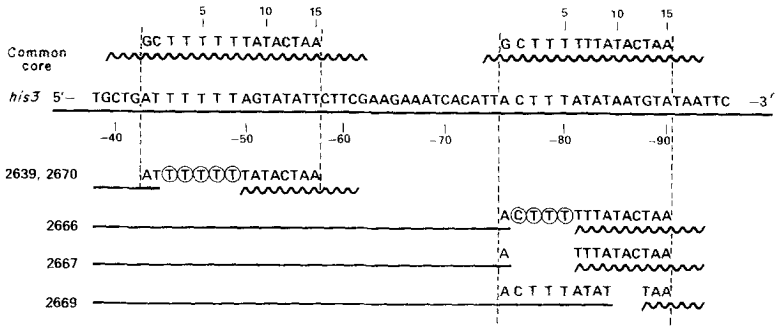

FIG. 2. Sequence analysis of 5 *int*-mediated deletion events. As illustrated in Fig. 1, all yeast DNA sequences are represented by a solid line while all λ sequences are represented by a wavy line. Only one strand of each nucleotide sequence is shown. The sequence is presented so that the 5′ to 3′ direction is from left to right and the orientation with respect to the normal λ map is preserved. The top line shows the nucleotide sequence of the core of *att* (determined by Landy & Ross, 1977). The second line indicates the DNA sequence located between 38 and 96 base-pairs before the start of the yeast *his3* structural gene (determined by Struhl & Davis, 1981). The left to right orientation of the *his3* sequence is the same as in λgt4-Sc2601 DNA. The λ and yeast DNA sequences are lined up as described in the text. The structures of the deletion mutants are shown below. Only the nucleotide sequence corresponding to the hybrid core region is shown explicitly; the flanking λ and yeast sequences are indicated by the solid and wavy lines. The presumed origins of the nucleotides at the hybrid cores are indicated; circled nucleotides could come from either yeast or λ DNA. Hyphens have been omitted from sequences for clarity.

sequence is arranged such that the best 1 : 1 correspondence is obtained between the novel joints, the common core, and the yeast DNA.

(b) *Sequence homology and the non-randomness of deletion break points*

A comparison of the 15 base-pair common core of the λ attachment site with the *his3* sequence immediately adjacent to each deletion endpoint reveals considerably more DNA sequence homology than expected on a random basis. A total of seven out of 15 nucleotides are homologous when the sequences are directly lined up; the number of matched residues expected on a random basis is 3·75. This suggests that partial sequence homology is important in formation of the deletions. Using a computer program developed by Korn *et al.* (1977) and modified by Doug Brutlag, a search for sequence homology between the core and the *his3* region was conducted. The following set of rather non-stringent homology parameters was used: the minimum number of matched nucleotides was five; at least 70% of the nucleotides in the prospective homology region had to be matched; "loop outs" up to three base-pairs were allowed; and after a mismatch, only one out of the next three nucleotides had to be matched. Even with this relaxed set of parameters, the 250 base-pair region upstream from the *his3* structural gene was "homologous" to the core in only two places (Figs 2 and 3). In the first case, the sequence T-T-T-T-T-A is present both in the core (nucleotides 3 to 9) and in the yeast sequence (−44 to −50). Deletion mutants λgt-Sc2639 and λgt-Sc2670 have break points within this sequence. In the second case, the region of homology is longer but less exact (Fig. 3). Deletion mutants λgt-Sc2666, λgt-Sc2667 and λgt-Sc2669 have deletion break points within this second region of homology. Further experiments confirm

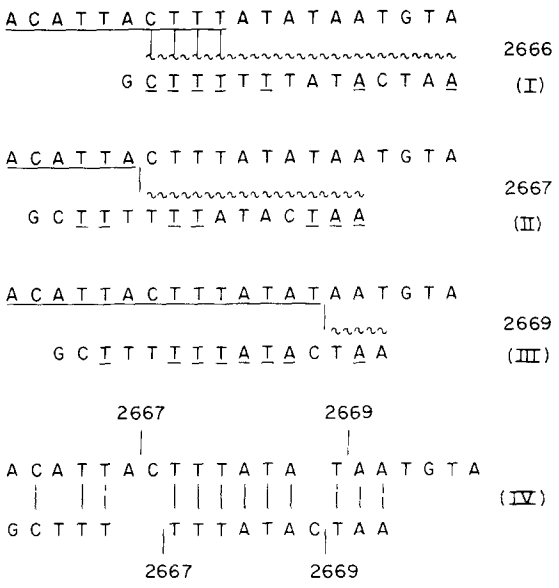

FIG. 3. Alternative pairing structures of yeast and λ DNA. Each structure shows pairing between *his3* nucleotides −71 to −98 (top line) and the core (bottom line). The recombinant sequences are indicated by the solid and wavy lines between the λ and yeast sequences. In all cases, underlined nucleotides indicate homology between yeast and λ DNA. Pairing structure I is drawn in an identical fashion to that in Fig. 1: the vertical lines indicate possible crossover points for λgt-Sc2666. Pairing structures II and III are obtained by sliding the core sequence with respect to *his3*. These are drawn such that the crossover does not result in deletion of DNA at the novel joint (as in Fig. 2). By this description, structure II accounts for the event generating λgt-Sc2669 and structure III accounts for that generating λgt-Sc2667. Structure IV is obtained after allowing loop-outs of up to 3 nucleotides. The break points of Sc2667 and Sc2669 (which are uniquely determined) are indicated by vertical lines. Hyphens have been omitted from sequences for clarity.

that these two yeast sequences most homologous to the core are hot spots for *int*-mediated events. Seventeen more independent *int*-generated deletions containing the intact *his3* structural gene but lacking some yeast DNA were isolated. Of these, 11 had deletion endpoints at either of the two positions observed in the mutants described above; six mapped indistinguishably from λgt-Sc2639 and five mapped indistinguishably from λgt-Sc2666. The other six deletion mutants had endpoints not within the 250 base-pair region of yeast DNA for which the sequence has been determined. This result does not reflect some artifact of mutant isolation because 15 other deletion mutants with break points scattered throughout this region have been isolated from a phage that cannot produce classical *int*-mediated events (Struhl, 1981).

(c) *Deletion break points occur at two or more sites within the core*

The positions of deletion break points within the core could provide mechanistic clues relating to *int* action *in vivo*. Determination of such break points requires a direct comparison of nucleotide sequences of the original parental and progeny DNA molecules. Because of the partial homology between the relevant yeast DNA

and the core, it is frequently impossible to localize the novel joint to the precise nucleotide. Nevertheless, at the novel joints, the deletion break points always occur internally within the core. In four mutants (λgt-Sc2639, λgt-Sc2666, λgt-Sc2667 and λgt-Sc2670) the break point occurs in the left part of the core (between nucleotides 2 and 7), while in λgt-Sc2669, the break point occurs in the right part of the core (between nucleotides 12 and 13).

(d) *Alternative outcomes of recombination with the same yeast DNA sequences*

In Figure 2 and in structure I of Figure 3, the deletion mutants are illustrated to show that λgt-Sc2666, λgt-Sc2667 and λgt-Sc2669 could arise from recombination between the core and the identical 15 base-pair sequence of yeast DNA. In this case, the hybrid core regions of these deletions differ in sequence even though the flanking sequences are identical. Two other configurations having seven or eight homologous base-pairs are obtained by sliding the common core sequence with respect to the yeast DNA sequence (Fig. 3, structures II and III). A hybrid structure in which 12 out of 15 core residues are paired with yeast nucleotides can be achieved by allowing loop-outs of up to two nucleotides (Fig. 3, structure IV). The significance of a given hypothetical structure depends upon its thermodynamic stability and its suitability as a substrate for recombination enzymes such as *int*.

(e) *The nature of the crossover points*

The usual view of recombination is depicted by total pairing of homologous parental molecules followed by strand cross-over between these molecules. The crossover occurs in a manner such that genetic information is not lost during the exchange. The deletion mutants result from recombination between sequences that are partially homologous.

For the deletion events represented by λgt-Sc2639, λgt-Sc2666 and λgt-Sc2670, a simple crossover between parental molecules can be drawn. However, when the relevant yeast DNA sequence is directly aligned with the core (as in Fig. 2), λgt-Sc2667 and λgt-Sc2669 appear to have deleted DNA in the vicinity of the novel joint. If the yeast and core DNA sequences are aligned such that the crossover between them does not result in the loss of genetic material (Fig. 3), it is impossible to draw a crossover at positions of homology. Therefore, the novel joints of λgt-Sc2667 and λgt-Sc2669 clearly have some unexpected features.

(f) *Hybrid core regions differentially affect* int*-mediated recombination*

The *int*-dependent recombination frequencies between λ phages are affected by the various kinds of attachment sites (POP′, BOB′, BOP′, ΔOP′ and POΔ′). Furthermore, variation in recombination frequency is observed in crosses involving different representatives of the same class of attachment site (Gottesman & Weisberg, 1971). The fact that deletion mutants of a given class (ΔOP′ or POΔ′) have different core regions (Hoess & Landy, 1978) suggests an explanation for the variation of recombinational frequency for these classes of *int*-dependent crosses; the results described below directly confirm this suggestion.

First, all five λ*his3* deletion phages (class ΔOP′) were crossed with a phage (λgt6-*ara*6) that contains the *b*522 deletion (class POΔ′) (Parkinson, 1971). From each cross, progeny phage were tested for *int* and *xis*. Half the progeny (those carrying *b*522) were *int*$^-$ *xis*$^-$; these were tested for *his3* function by the lytic plaque assay. The *int*$^-$ *xis*$^-$ *his3*$^+$ phages represent recombinants of the ΔOΔ′ variety. The frequency of this event has been reported previously as 1% (Gottesman & Weisberg, 1971). However, only λgt-Sc2667 recombines with λgt-*ara*6 at this frequency. The other λ*his3* deletion phages produce equivalent recombinants at a frequency of less than 10^{-3}.

This differential recombination frequency is more dramatically indicated when the λ*his3* phages are crossed with λ1096, a phage that contains a deletion of the POΔ′ class (*b*1319). Because of the phages used in the cross, it is possible to directly select for recombinants as rare as 10^{-8}. The frequencies vary over almost four orders of magnitude (Table 1). The most frequent of these recombination events again occurs with λgt-Sc2667, but about 20 times more infrequently than the analogous cross with *b*522. The most infrequent of these recombination events (with λgt-Sc2669) occurs at 2×10^{-7}, a frequency about 100 times less than that of *int*-mediated deletion formation.

TABLE 1

int-*mediated recombination frequencies for various POΔ′-ΔOP′ crosses*

λ*his3* phage	*b*522	*b*1319	*bio*256
λgt-Sc2639	$<10^{-3}$	2×10^{-6}	1×10^{-3}
λgt-Sc2666	$<10^{-3}$	1×10^{-5}	2×10^{-3}
λgt-Sc2667	1×10^{-2}	5×10^{-4}	5×10^{-2}
λgt-Sc2669	$<10^{-3}$	2×10^{-7}	1×10^{-5}
λgt-Sc2670	$<10^{-3}$	5×10^{-6}	5×10^{-4}
λgt-Sc2694	$<10^{-3}$	$<10^{-8}$	$<10^{-8}$

The 5 λ*his* deletion mutants (ΔOP′) and a λ*his3* mutant lacking the entire attachment site (λgt-Sc2694) are listed in rows. The 2 POΔ′ phages (containing *b*522, *b*1319) and the POB′ phages (containing *bio*256) are listed in columns. The frequencies of the ΔOΔ′ recombinants for each of these crosses are shown at the intersection of the appropriate row and column.

Recombination of the λ*his3* phages with the *bio*256 substitution (class POB′) occurs at approximately 100 times the frequency as compared to recombination with *b*1319. Again, the recombination frequency with different derivatives varies over four orders of magnitude (Table 1).

Therefore, phages that differ only in their hybrid core sequences behave extremely differently in *int*-mediated recombination. It is of particular interest to note that the relative ability of individual λ*his3* derivatives to recombine with a number of different hybrid cores is roughly constant.

(g) *Hybrid core regions differentially affect* his3 *expression*

The yeast *his3* gene codes for imidazoleglycerolphosphate dehydratase. In *E. coli*, functional expression of *his3* results in the complementation of histidine

auxotrophs lacking the bacterial imidazoglycerolphosphate dehydratase (Struhl *et al.*, 1976; Struhl & Davis, 1977). This complementation is observed in two ways (Struhl *et al.*, 1980). During lytic growth, phenotypically detectable *his3* expression depends upon readthrough transcription from the λ promoter P_L. On the other hand, *his3* expression from integrated hybrid prophages depends upon a promoter in yeast DNA because transcription from any of the major λ promoters (P_L, P_R and $P_{R'}$) is repressed. This "yeast in coli" promoter was allegedly mapped by analysing both modes of *his3* expression in many of the λ*his3* deletion mutants including those described here. Deletion mutants λgt-Sc2694 and λgt-Sc2695 retain the structural gene but do not express *his3* as prophages because they lack this promoter. Their deletion break points within *his3* occur between 44 and 46 base-pairs upstream from the start of the structural gene (Struhl & Davis, 1981). However, deletions λgt-Sc2639 and λgt-Sc2670, with deletion endpoints between 45 and 50 base-pairs prior to the structural gene, express *his3* under the same conditions (Struhl & Davis, 1980; Table 2). In other words, two sets of deletion mutants with nearly the same yeast DNA endpoint but with different λ DNA endpoints express *his3* differentially. Because the wild-type *his3* sequence in this region (Struhl & Davis, 1981) reveals no sequence that resembles those of 46 *E. coli*

TABLE 2

Lysogenic his3 *expression*

Phage	1	2	3
λgt-Sc2639	+	+	Lawn
λgt-Sc2666	+ +	+ +	Lawn
λgt-Sc2667	+	+	Lawn
λgt-Sc2669	+ +	+ +	Lawn
λgt-Sc2670	+	+	Lawn
λgt-Sc2671	+	+	+
λgt-Sc2694	−	−	−
λgt-Sc2667 *b522*	+	NT	−
λgt-Sc2639 *b1319*	−	NT	−
λgt-Sc2666 *b1319*	±	NT	−
λgt-Sc2667 *b1319*	−	NT	−
λgt-Sc2669 *b1319*	+	NT	Lawn
λgt-Sc2670 *b1319*	−	NT	−
λgt-Sc2639 *bio256*	+	NT	+
λgt-Sc2666 *bio256*	+ +	NT	+ +
λgt-Sc2667 *bio256*	+	NT	+
λgt-Sc2669 *bio256*	+ +	NT	+ +
λgt-Sc2670 *bio256*	+	NT	+

The left-most column lists the phages used in these experiments (see Fig. 4). Each column describes a particular way in which the expression was assayed as follows (these correspond to methods 1 to 3 as described in Materials and Methods): (1) direct selection after infection into a wild-type lysogen; (2) assay after selection for single lysogens; (3) direct selection after infection into non-lysogen. For lysogenic expression, + + indicates growth in the presence of 1 mM-aminotriazole, + indicates growth in the absence of histidine but not in the presence of aminotriazole, ± indicates slower than normal growth (see text), and − indicates no growth. In some cases, a confluent lawn of bacteria was produced, probably the result of increased lytic expression. This lawn is eliminated by the addition of 5 mM-sodium pyrophosphate, a condition that prevents phage re-infection; the original λ*his3* derivatives then produce His^+ colonies. NT, not tested.

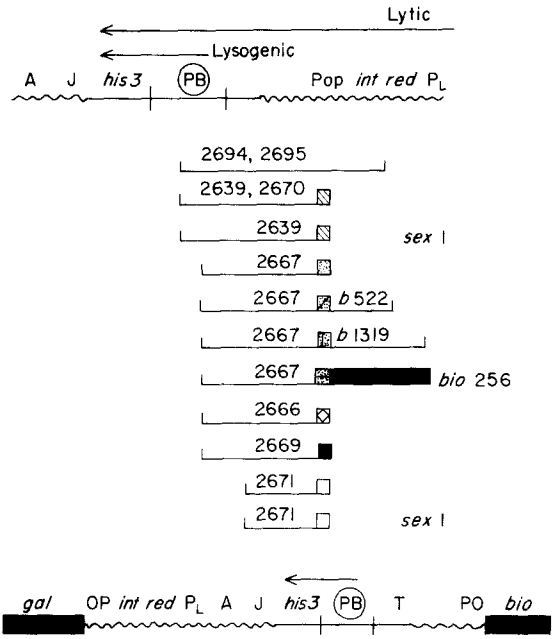

FIG. 4. Lytic and lysogenic expression of λ*his3* derivatives. The important genetic elements of λgt4-Sc2601 are indicated above the wavy–straight hybrid line (not drawn to scale). PB indicates the Pribnow box presumed to be essential for lysogenic *his3* expression in *E. coli*, and T indicates the site of the proposed terminator for readthrough transcription (see text). Lytic expression of *his3* depends upon readthrough transcription initiated from the λ promoter P_L (Struhl *et al.*, 1980). The location of the presumed transcripts necessary for lytic and lysogenic expression are indicated above λgt4-Sc2601. The structures of phages used in this work are diagrammed below the wild-type sequence. The boxes indicate hybrid core regions for each of the *int*-mediated deletion mutants; different hybrid cores are indicated by different symbols. The *b*522, *b*1319 and *bio*256 derivatives are indicated only for λgt-Sc2667; the hybrid cores of these derivatives may differ from λgt-Sc2667. The bottom line indicates the structure formed by prophages integrated at the normal bacterial attachment site, the positions of the *gal* and *bio* operons are shown.

promoters (reviewed by Rosenberg & Court, 1979), *his3* expression in λgt-Sc2639 is probably initiated from a promoter mapping between *att* and 0·633 on the λ map. The only known promoter in this region (P_{int}) is the *cII* and *cIII*-dependent promoter in *xis* from which the *int* gene is transcribed (Shimada & Campbell, 1974). To determine whether P_{int} is responsible for *his3* lysogenic expression in λgt-Sc2639 and the other derivatives, it is necessary to remove it by deletion or to separate it from *his3* sequences by *int*-mediated recombination. The technical complications for these experiments are described in detail in Materials and Methods. The results are presented in Table 2 and summarized below.

(1) By the model of Campbell (1962), lysogens with a single λ*his3* prophage should sever *his3* sequences from P_{int} and instead link them to *bio* sequences (see Fig. 4). For each tested phage, 50 independent lysogens isolated under conditions strongly favouring single lysogeny at the normal bacterial attachment site all express *his3* (Table 2, column 2).

(2) The identical *his3-bio* phages were constructed by crossing the λ*his3* deletions

with *bio*256. Though these *bio*256 derivatives do not contain P_{int}, they integrate at the same site in the *E. coli* chromosome by general recombination *via bio* homology. In these cases, all the phages express *his3* (Table 2, column 3). Therefore, the unexpected lysogenic expression of λgt-Sc2639 (and related phages) occurs in the absence of P_{int}.

(3) When *his3* is linked to *bio* by either of the methods described above, lysogens of λgt-Sc2666 and λgt-Sc2669 grow in the presence of aminotriazole, while those of λgt-Sc2639, λgt-Sc2667, λgt-Sc2670 and λgt-Sc2671 do not. This expression is not due to readthrough transcription from the biotin operon because it is not affected by addition of biotin to the medium (conditions that severely decrease such transcription). Thus, λgt-Sc2666, λgt-Sc2667 and λgt-Sc2669, though identical in sequence except for the core, express *his3* at different levels.

(4) When P_{int} was also removed by either the *b*1319 or the *b*522 deletion, the *b*1319 derivatives of Sc2639, Sc2667 and Sc2670 do not produce His^+ colonies, the *b*1319 derivative of Sc2669 and the *b*522 derivative of Sc2667 express *his3* normally, while that of Sc2666 produces a lowered number of slow growing colonies.

4. Discussion

(a) *Some comments concerning* int*-mediated recombination*

The model for *int*-mediated recombination proposed by Shulman & Gottesman (1973) invokes joining of staggered ends of DNA in a manner analogous to covalent joining of ends produced by restriction endonucleases. Such a mechanism requires two nicking events that occur on opposite strands and at two distinct locations. The fact that *his3* deletion break points occur at two or more locations within the core is consistent with this model. From the sequences of some classic *int*-mediated deletions of λ, Hoess & Landy (1978) came to the same conclusion; in those cases, however, the crossover point could not be determined. These results do not exclude the possibility that *int* does not nick at a specific location of the core or that the apparently different break points could be produced by repair processes following nicking at one unique site.

It has been known for quite a while that the core is not the only sequence that influences *int*-mediated recombination (Gottesman & Weisberg, 1971). In particular, sequences to the right of the core of the normal phage attachment site seem to be important both genetically and biochemically (Ross *et al.*, 1979). However, λgt-Sc2639 contains the exact core sequence of λ*b*511 (Hoess & Landy, 1978) and the identical P′ arm, yet it recombines at considerably lower frequency with *b*522 (Gottesman & Weisberg, 1971; unknown referee, personal communication). This suggests that sequences to the left of the core are important.

(b) *Formation of deletions*

There are two general classes of mechanisms by which *int*-mediated deletion mutants could be formed. Deletions could arise by nicking both the common core and yeast DNA and joining the ends. In this case, the low frequency of formation would be explained by a lowered efficiency of nicking yeast DNA by *int* protein. On

the other hand, deletions could be formed by nicking the core followed by single strand invasion of λDNA into duplex yeast DNA in a manner analogous to that proposed for general recombination (Holliday, 1964); here, the low frequency would result directly from poor homology between yeast DNA and the core. By this second model, the second strand break in yeast DNA would probably not be *int*-dependent. Either of the two explanations would explain the observation that different hybrid core regions behave differently in *int*-mediated recombination.

I suggest that the results presented here favour the view that *int*-mediated deletions are formed by *int* action at both the core and yeast DNA. The best evidence is that individual *λhis3* hybrid phages have characteristic *int*-mediated recombination frequencies. The *att* site of λgt-Sc2667 recombines relatively well with two different POΔ′ sites (*b*1319 and *b*522) and POB′ (*bio*256) while the *att* site of λgt-Sc2669 recombines about 10^4-fold less efficiently in the cases determined. The other *λhis3* deletions, in all cases tested, recombine at intermediate frequencies. If *int*-mediated deletions are formed by nicking at the normal λ attachment site followed by strand displacement of yeast DNA, one would expect recombinant frequencies from particular crosses to depend upon the quality of DNA sequence homology between pairwise combinations. In fact, in cases for which the sequences are known (*b*522 and *bio*256; Landy & Ross, 1977; Hoess & Landy, 1978) there is no correlation between the degree of homology and the frequency of recombination. For example, the *his3* core sequences of λgt-Sc2639 and λgt-Sc2666 have 13 or 14 contiguous base-pairs of homology to the wild-type core of *bio*256, yet these crosses produce recombinants at a significantly lower frequency than that obtained with λgt-Sc2667 (which has only a subset of this homology). It is important to remember that three of the *λhis3* deletions are identical in sequence except at the hybrid core region. Thus, it is likely that the frequency of particular *int*-mediated recombination events depends upon *int* action at both recombining core sequences.

A surprising result of this work is that in two out of five cases recombination between partially homologous sequences generates new combinations of genetic material that do not result from simple homologous crossing over. In over 100 unselected recombination events within a very small interval of the yeast chromosome, no genetic evidence of unusual (mutable) recombination has been observed (Fogel & Mortimer, 1969). The unusual structures at the novel joints might be explained by non-homologous crossing over or by imperfect repair. If, as suggested above, deletions are formed by joining the staggered ends of the wild-type core and a yeast DNA sequence that is effectively a mutant core, one would expect that the protruding ends would differ in sequence. Thus, finding unusual repair in two out of five events might not be so surprising.

(c) *Core sequences and promoter function*

Two lines of evidence suggest that the core can supply sequences that constitute part of a promoter for *his3* expression. The first is that all the *λhis3* phages, when integrated into the normal bacterial attachment site by *int*-mediated recombination under conditions unlikely to result in double lysogeny, express *his3*. In these cases, *his3* is separated from P_{int} by the recombination event and is now

directly fused to *bio* sequences. The *bio*256 derivatives integrated by *bio* homology should be equivalent to the above single lysogens, and indeed they give the same results. This clearly establishes conditions for lysogenic *his3* expression in the absence of P_{int}. The second line of evidence is that when *his3* is fused to *bio* by either of the above ways, the level of expression depends upon the core sequence. This can result either because changes in the core affect transcriptional initiation, or because changes in the core alter transcriptional termination, and hence different levels of readthrough transcription. However, the core sequences are unlikely to be sites of termination because they do not contain sequences of hyphenated, dyad symmetry, runs of T residues in the mRNA coding strand, and they are not G+C-rich, all features of *E. coli* terminators (Rosenberg & Court, 1979). Thus difference in *his3* expression in Sc2666, Sc2667 and Sc2669 almost certainly results from promotion rather than termination effects.

In this regard, the core sequence provides a good fit to the Pribnow box, an important promoter element in *E. coli* (Pribnow, 1975). The wild-type core contains the sequence 5′-T-A-G-T-A-T-3′ reading from right to left (see Table 3), while the canonical Pribnow box is T-A-T-A-A-T (Rosenberg & Court, 1979). In particular, the four homologous nucleotides in the core are those most highly conserved among 46 *E. coli* promoters.

TABLE 3

Hypothetical Pribnow box sequences at hybrid core regions

Deletion mutant	Hybrid core sequences		*his3* level
	Pribnow box	Initiation site	
λgt-Sc2639	T-A-G-T-A-T	A-A-A-A-A-A-A-T-C-A-G-C-A-G	+
λgt-Sc2670	T-A-G-T-A-T	A-A-A-A-A-A-A-T-C-A-G-C-A-G	+
λgt-Sc2667	T-A-G-T-A-T	A-A-A-T-A-A-T-G-T-G-A-T-T-T	+
λgt-Sc2666	T-A-G-T-A-T	A-A-A-A-A-A-G-T-A-A-T-G-T-G	+ +
λgt-Sc2669	T-A-A-T-A-T	A-A-A-G-T-A-A-T-G-T-G-A-T-T	+ +
	T-A-A-A-G-T	A-A-T-G-T-G-A-T-T-T-C-T-T-C	
his3 wild-type	T-A-A-A-G-T	A-A-T-G-T-G-A-T-T-T-C-T-T-C	+
Common core	T-A-G-T-A-T	A-A-A-A-A-A-G-C	
Pribnow box	T-A-T-A-A-T		

The nucleotide sequences of the *his3* "sense" strands of the *int*-mediated deletion mutants are indicated such that the 5′ to 3′ direction is from right to left. Because the *his3* gene is transcribed from right to left in λgt4-Sc2601, these nucleotides represent the opposite strand and the opposite direction from the sequences shown in Figs 2 and 3. The nucleotide sequence is divided into 2 parts. The first consists of the Pribnow box; the second includes 10 nucleotides past the Pribnow box. The wild-type *his3* sequence and the canonical Pribnow box are also shown.

How do different hybrid cores affect expression? Sc2669 might increase expression because its Pribnow box may be better or because of two alternative boxes in the same region (see Table 3). The other deletions all have the same Pribnow box, but their sequences differ around the site of transcriptional initiation predicted by the Pribnow box. The nucleotides at the points of difference should interact with RNA polymerase (Siebenlist *et al.*, 1980), but it is not clear why the Sc2666 sequence seems to be better.

Hybrid core sequences do not always produce *his3* expression. The *b*1319 derivatives of Sc2639, Sc2667 and Sc2670 are His$^-$ and *his3* expression of Sc2666 *b*1319 seems to be reduced. This can be explained in two ways. Because these derivatives are produced by *int*-mediated recombination between partially homologous cores, it is likely that the recombinants have even more novel core structures; in some cases, these may eliminate the putative Pribnow box. On the other hand, the *b*1319 derivatives fuse sequences upstream from the core that are different from the *bio* region. Sequences centered around 35 base-pairs upstream from the RNA initiation point are important for promoter function (Gilbert, 1976). In the dilysogen experiments indicating *his3* function for λgt-Sc2667 *b*522 and for λgt-Sc2669 *b*1319 (Table 2, column 1) it may be hard to distinguish promotion from integration effects (see Materials and Methods).

Transcription from hybrid core sequences or readthrough transcription from any other promoter such as P_{int} is only effective for expressing *his3* if the transcript is not terminated. The facts that (1) Sc2639 *sex*1 but not Sc2671 *sex*1 produce detectable lytic *his3* expression; (2) single infection of Sc2639 but not Sc2671 into non-lysogenized cells produces a lawn instead of lysogens; and (3) expression of Sc2666 and Sc2669, but not Sc2671, as single prophages integrated at the normal attachment site allow for growth in the presence of aminotriazole all suggest that a terminator for readthrough transcription maps between the endpoints of Sc2669 and Sc2671.

(d) *Relationship between recombination and gene expression*

A relationship between recombination and gene expression was initially proposed by McClintock (1956) to explain the behaviour of controlling elements in maize. The *E. coli* insertion element IS2 can also affect gene expression (Saedler *et al.*, 1974). The sequences at novel joints within *Drosophila melanogaster* histone gene repeat units are similar to a sequence proposed to be an element of a eukaryotic promoter (Goldberg, 1979). And, transcription of retroviruses (Hughes *et al.*, 1978) and transposable elements in *Drosophila* (G. M. Rubin, personal communication) or in yeast (R. Elder & R. W. Davis, personal communication) is initiated in the end repeat sequences thought to be important for site-specific recombination. In this regard, it is interesting that the core seems under certain circumstances to supply a Pribnow box necessary for *his3* expression.

A relationship between recombination and gene expression would be quite beneficial for evolutionary adaptation. A recombination event could result in control of a particular structural sequence by a new regulatory sequence. Sites of *int*-mediated deletions are favoured by sequence homology to the core, and the core has homology to the Pribnow box, a key element of a prokaryotic promoter. In addition, illegitimate *int*-mediated recombination generates new genetic combinations at the base-pair level. Taken together, it seems likely that such events could easily result in altered gene expression by virtue of unusual sequences at the novel joint. The hybrid core regions of λgt-Sc2666, λgt-Sc2667 and λgt-Sc2669 seem to be responsible for different levels of *his3* gene expression. Though phage λ and *E. coli* cells do not normally contain the yeast *his3* gene, it is clear that

simple recombination events can generate a set of related derivatives some of which respond to specific environmental conditions.

(e) *Caveats about using deletions for mapping point mutants and promoters*

Deletion mutations are frequently used to map promoter regions on the assumption that sequences missing in non-functional mutants are important. The converse assumption is that functional derivatives contain all promoter sequences. Previous experiments demonstrated that a yeast DNA sequence acts as a promoter to express the *his3* gene in *E. coli* (Struhl *et al.*, 1976,1980; Struhl & Davis, 1980). However, mapping the *his3* promoter has been complicated by fusion of P_{int} in certain derivatives, by the hybrid core sequences, by termination of readthrough transcription, and by the process of integration which produces undesired recombinants. While many of these complications are specific to the case at hand, others are likely to be general problems. The possibility of fused promoters and transcription terminators must be considered in *E. coli* particularly as translation can be initiated efficiently on many mRNA templates.

In addition, the fact that sequences at the novel joints may be unusual could affect significantly the mapping of point mutants by recombination with deletion mutants. For example, crosses between a given deletion mutation and a set of point mutations mapping at the novel joint may or may not produce recombinants with wild-type sequences. For a given cross, the result will depend upon whether the novel joint sequence is altered at the same nucleotide as the point mutation. Extensive deletion mapping of the *Salmonella typhimurium* histidine operon has produced data that may be explained in such a manner (Hoppe *et al.*, 1979).

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