Attachment Site of the Genetic Element e14

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The *Escherichia coli* K-12 genetic element, e14, contains a 216-base-pair region that is homologous to a portion of the host chromosome. This region serves as the integration site for the element. The 216-base-pair homology is interrupted by 28 mismatches distributed through the sequence. The actual integrative crossover occurs within the first 11 base pairs from one end of the region. To test factors which affect e14 site-specific recombination, we cloned the attachment sites of free e14 and the host chromosome into the same plasmid. The cloned attachment sites recombined intramolecularly in a process that required the presence of a chromosomal copy of e14 in the host cell as well as the induction of SOS. Recombination events that mimicked both integration and excision occurred under the same conditions and to roughly the same extent.

The genetic element e14 is a nonessential constituent of the *Escherichia coli* K-12 chromosome (6). It determines several phenotypes of K-12, including the Pin inversion system (8, 17), the SfiC suppressor of filamentation (11), the McrA restriction of 5-methylcytosine DNA (E. A. Raleigh, personal communication), and the Lit system affecting T4 late gene expression (L. Snyder, personal communication). Certain properties of the element suggest that it is a prophage. For example, after SOS induction, it is excised from the chromsome as a 14.4-kilobase (kb) DNA circle (6). Furthermore, it can reintegrate at the same site independently of the host RecA system (4). However, we have not observed induced cultures to produce plaque-forming particles, and we have not observed e14 DNA to replicate independently either after excision or upon transformation of a cured recipient (4; H. Brody and C. Hill, unpublished data). Thus, e14 may be a defective phage or it may belong to some other class of genetic element.

Earlier work suggested that two aspects of the e14 site-specific recombination system are unusual. First, it excised slowly and/or inefficiently after SOS induction (6), and, second, Southern blotting experiments suggested that free e14 and the host chromosome share substantial homology at the site of attachment (4). In this report we describe the nucleotide sequence of the e14 site-specific recombination sites and other properties of the system.

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacterial strains and culture conditions have been described previously (4). The symbols (e14⁺) and (e14⁰) refer to bacterial strains with and without e14 integrated into the host chromosome. The symbols *attE*, *attC*, *attL*, and *attR* refer to the attachment regions of free e14, the host chromosome, and the left and right ends of integrated e14, respectively. Recombinant plasmids are described in Fig. 1 and Fig. 2. Construction of pHB106 and pHB108 was described previously (4). To make pHB109, the 2.3-kb *Eco*RI-*Ava*I fragment from pHB106 was subcloned into the *Eco*RI site of pBR325 (3) after an *Eco*RI linker (purchased from New England BioLab, Inc.) was ligated to the *Ava*I end. The construction of pHB206 was as

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follows. Plasmid pHB109 was forced to integrate into the chromosome of the *polA1* (e14⁰) mutant, CH1493, by using the homology provided by the insert (6). This integration produced a transformant which had the pBR325 vector inserted into the chromosome near *attC*, between direct repeats of the pHB109 insert (Fig. 1b). Next, chromosomal DNA from this transformant was digested with *Hin*dIII, which cut at the *Hin*dIII site within the integrated vector and at the *Hin*dIII site to the left of *attC*. This fragment contained *attC*, the vector origin, and the Ampʳ locus. Closure of the circle by ligation resulted in pHB206 which contained the *attC* region. To construct pRE-I and pRE-D (Fig. 2), the 3.3-kb *Eco*RI fragment containing *attC* from pHB206 was inserted into the *Eco*RI site of pHB108; pRE-I and pRE-D differed by the orientation of this insert.

DNA isolation and analysis. Plasmid extraction was done by the alkaline lysis procedure of Birnboim and Doly (2), except that the preparations were treated with 0.5% diethylpyrocarbonate for 15 min at 65°C after the neutralization step to inhibit exonucleases (modification communicated by R. Baker). Conditions for extraction of genomic DNA, restriction endonuclease digestion, ligation, and gel electrophoresis were described previously (4).

DNA sequencing. Restriction fragments containing the attachment regions from pRE-I and pRE-X were made blunt ended by using the Klenow fragment of DNA polymerase (12) and then ligated into the *Sma*I site of pUC19 (14). A series of deleted inserts was made by the exonuclease III procedure of Henikoff (7). Sequencing was done by the Maxam-Gilbert procedure (13) as described by Bencini et al. (1). Both strands of the reported sequences were determined either by a combination of 5' and 3' terminal labeling (1) or by sequencing overlapping regions from opposite directions.

RESULTS

Site-specific recombination between cloned attachment regions. In earlier work (4, 6), e14 excision and reintegration were detected by Southern analysis. This technique was not suitable for the type of study to be described here, and so an assay with cloned attachment sites had to be developed. Comparison of the restriction maps of free and integrated e14 had indicated that the crossover occurred within the 0.95-kb *Hin*dIII-*Ava*I segment of free e14 and that this segment also contained the substantial homology shared by free e14 and the cured chromosome (4). Therefore, we made
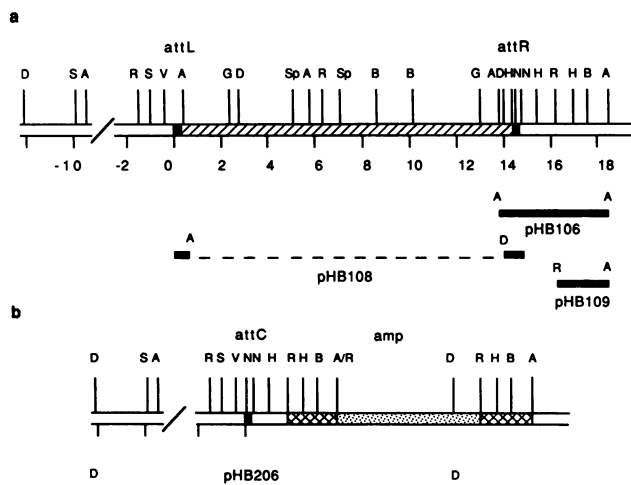
FIG. 1. Restriction map of the *E. coli* chromosomal region containing e14. (a) Symbols: ▨, integrated e14 element; ▭, host chromosome (the *purB* locus is to the left of e14, and *umuC* is to the right [4]); ▬, left- and right-hand termini of e14 (*attL* and *attR*, respectively). Coordinates are in kilobases. The inserts of relevant recombinant plasmids are shown below the map. Note that the insert of pHB108 is the *Hin*dIII-*Ava*I fragment of excised e14 containing *attE*. Restriction site designations: A, *Ava*I; B,*Bam*HI; D, *Hin*dIII; G, *Bgl*III; H, *Hpa*I; N, *Nde*I; R, *Eco*RI; S, *Sal*I; Sp, *Sph*I; V, *Pvu*II. Additional *Eco*RI, *Bgl*II, *Hpa*I, *Nde*I *Pvu*II, and *Sph*I sites in the region have not been mapped. (b) Structure formed by the integration of pHB109 into a *polA* (e14°) strain. Symbols: ▨, vector DNA; ▨, duplicated host DNA. The plasmid pHB206 was generated as described in the text.

the tentative assumption that the functional attachment sites of the element (*attE*) and the host (*attC*) would involve these homologies. Two recombinant plasmids were constructed which had DNA fragments containing the homologies from both e14 and the cured host chromosome inserted into the same pBR325 vector (Fig. 2). The two plasmids, called pRE-I and pRE-D, differed in the relative orientation of the inserts. The orientation of the fragments in plasmid pRE-I was such that recombination between *attE* and *attC* within the same molecule would invert the segment between them if the attachment sites paired with the same alignment as during integration. It was anticipated that this recombination could be detected by appropriate restriction endonuclease digests of extracted plasmid. The plasmid pRE-D was identical to pRE-I, except that the *Eco*RI fragment containing *attC* was in the opposite orientation. Consequently, recombination between *attE* and *attC* of pRE-D would be expected to resolve the plasmid into two smaller circles, only one of which would have an origin of replication (Fig. 2).

We wished to determine whether recombination between these subcloned attachment sites would occur and, if so, whether the recombination depended on e14-encoded functions. To do this, pRE-I was introduced by transformation into a pair of isogenic *recA441* strains which differed by the presence or absence of an integrated copy of e14. The cultures were grown to stationary phase at 32°C and then diluted 1,000-fold into medium at 42°C to cause SOS induction; controls were similarly diluted, but at 32°C. Following dilution, the cultures were incubated for 5 h. Plasmid was extracted from the cultures and digested with a combination of the restriction enzymes *Hin*dIII and *Ava*I to cut pRE-I into fragments of 7.6 and 0.95 kb (Fig. 3, lane 1). We

observed that if the host cell contained e14 and was SOS induced, bands of 5.4 and 3.3 kb appeared in addition to the bands expected from pRE-I (lane 4). These new bands were of the size expected if inversion between the two attachment sites had occurred in part of the plasmid population; they were not observed if the host cell lacked e14 (lane 2) or was not induced for SOS (lane 3). This showed that the chromosomal copy of e14 was supplying a function(s) essential for the site-specific recombination and that at least one component of this reaction required SOS induction for expression. A similar experiment with pRE-D gave analogous results, namely, that pRE-D was resolved into two circles of the expected size if the host cell contained a chromosomal copy of e14 and was SOS induced (data not shown). Implicit in the above results is the conclusion that in accord with our original assumption; functional *attE* and *attC* sites are contained in the respective inserts.

Recombination between the *att* sites contained in pRE-I mimicked the integration of e14 into the chromosome. We next wished to determine whether recombination mimicking excision could occur. We first needed a suitable test plasmid. To obtain this plasmid, plasmid preparations from a pRE-I culture that had undergone recombination (Fig. 3, lane 4) were used to transform a *recA56* recipient (CH1333), and the Amp^r transformants were screened to determine whether they carried pRE-I or whether they had acquired a rearranged derivative of pRE-I. Several rearranged derivatives
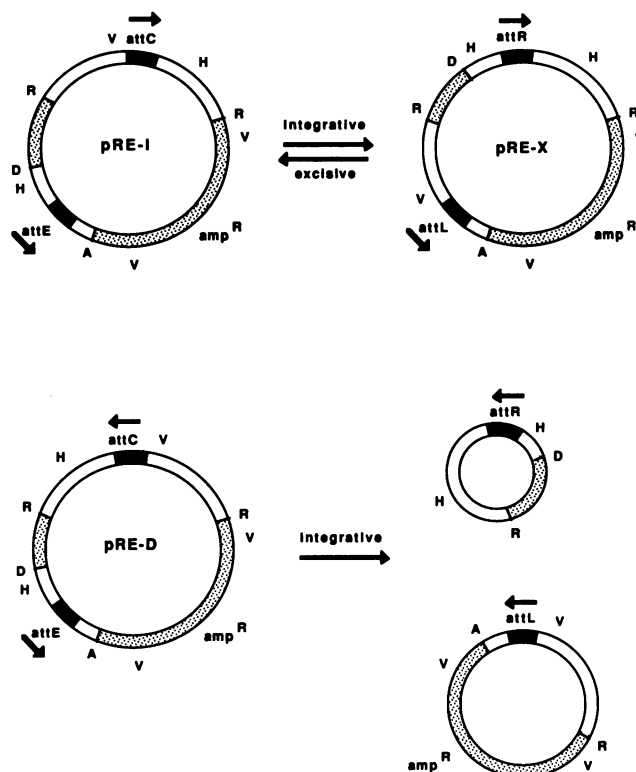

FIG. 2. Structures of recombinant plasmids containing the e14 attachment sites. Plasmid pRE-I contains *attE* and *attC* in an orientation such that intramolecular recombination between them would cause an inversion. Plasmid pRE-D is similar, but the *attC* insert is in the opposite orientation. Intramolecular recombination within pRE-D would resolve the plasmid into two circles. Symbols: ▭, insert DNA; ▬, attachment sites; ▨, vector DNA. Designation of restriction sites is as indicated in the legend to Fig. 1.
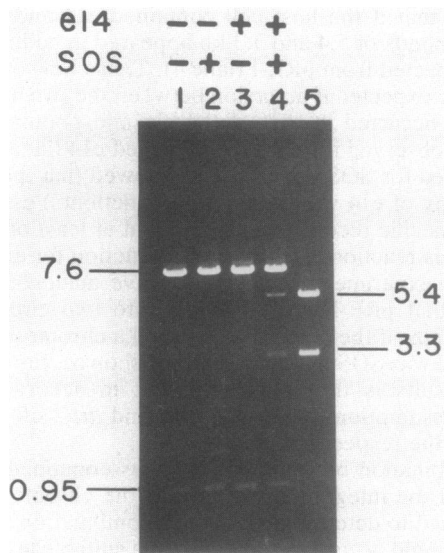
FIG. 3. Site-specific recombination within pRE-I and pRE-D. Plasmid extraction, digestion with HindIII and AvaI, and electrophoresis were as specified in Materials and Methods. CH1035 (recA441 e14⁰) cultures containing pRE-I were grown at 32°C (lane 1) or 42°C (lane 2); CH826 (recA441 e14⁺) cultures containing pRE-I were grown at 32°C (lane 3) or 42°C (lane 4) as described in the text. Lane 5 shows a HindIII-AvaI double digest of pRE-X, isolated from the plasmid preparation used in lane 4.

were isolated, and a HindIII-AvaI digest of one, called pRE-X, is shown in Fig. 3, lane 5. The pRE-X plasmid should contain the two prophage ends, attL in the 5.4-kb interval and attR in the 3.3-kb interval. To substantiate that the recombination event changing pRE-I to pRE-X had actually occurred in the region of the attachment sites, the plasmids, along with genomic DNA from an (e14⁺) strain, CH734, were digested with PvuII and AvaI and then subjected to Southern blot analysis. After probing with the 0.95-kb insert of pHB108 (containing attE), we observed that pRE-X had acquired a 0.85-kb interval identical to the attL fragment from the genomic digest.

To test whether the reverse reaction mimicking e14 excision could occur, the experiment described in Fig. 3 was repeated with pRE-X. We found that after prolonged SOS induction, plasmid extracted from an (e14⁺) culture initially containing pRE-X yielded a mixture of pRE-X and pRE-I. As above, both the presence of a chromosomal copy of e14 and SOS induction were required for the plasmid recombination. Thus, recombination in both directions, mimicking either integration or excision, appeared to occur under the same conditions. The courses of these opposing reactions were compared directly in the experiment shown in Fig. 4. The protocols for this experiment and that shown in Fig. 3 were similar, except that for this experiment, after transfer to 42°C the cultures were maintained at an A_{590} between 0.1 and 0.5 by serial dilution. We found that for both pRE-I (Fig. 4, lanes 1 through 6) and pRE-X (lanes 7 through 12), the amount of rearranged plasmid increased steadily, and after 7.5 h both cultures were approaching equilibrium; i.e., roughly equal amounts of pRE-I and pRE-X were present regardless of the starting plasmid (compare lanes 6 and 12). It was apparent that under these conditions, both the integrative and excisive recombination reactions proceeded with similar rates.

Sequence of the attachment regions. As noted above,

Southern blot analysis had suggested that e14 attC and attE share considerable homology. To determine the nature of this homology and its role in e14 site-specific recombination, we subcloned and sequenced the regions containing attC and attE from pRE-I as well as several independent regions of attL and attR produced from pRE-I by inversion. The sequence shown in Fig. 5 for attE extends 439 bp rightward from a HpaI site (bp 14350 in Fig. 1a). The sequence shown for attC extends 439 bp leftward from a NdeI site (bp 14790 in Fig. 1a). Comparison of the two regions revealed that they share a 216-bp segment of homology, differing at 28 positions within the homology. Sequence determination of two independently generated attL sites and two attR sites showed that the crossover had occurred within this region of homology. That is, the two attL fragments were identical to attC leftward of the 216-bp homology region and identical to attE rightward of the homology. The reciprocal was true of the two attR fragments. Furthermore, the position of the crossover could be located by examining attL and attR with respect to the 28 mismatches between attC and attE. In all four independent cases, the crossover had occurred between the left end of the homology and the first mismatch 12 bp into the homology. This exchange region is indicated in Fig. 5.

DISCUSSION

Previous work has shown that e14 can integrate into the host chromosome independently of host recA or recB functions (4) and that excision of e14 is induced by SOS induction (6). Together, these observations suggested the existence of a site-specific recombination system that is normally repressed. A similar conclusion has been drawn from zygotic induction experiments (17). The experiments shown in Fig. 3 and 4 showed that this site-specific recombination can occur between e14 and host sites cloned into the same plasmid and that this recombination is dependent on an e14-encoded function activated by SOS induction. The experiments shown in Fig. 4 monitored the time course of both integrative- and excisive-type reactions and illustrated two features of e14 site-specific recombination: its slowness or low efficiency and its apparent lack of directionality. The low efficiency of the excisive recombination was in agreement with earlier observations that 2.5 h or more was required to
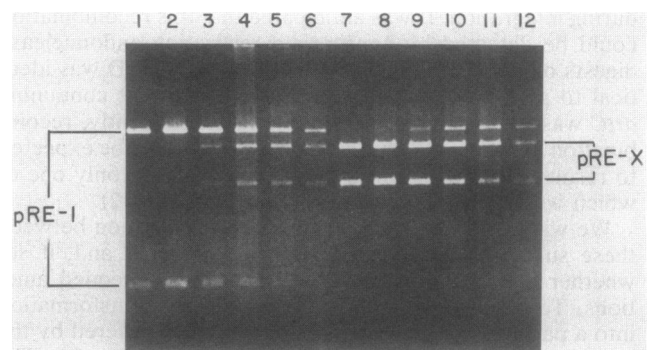
FIG. 4. Comparison of the integrative and excisive recombination reactions. Procedures were generally as described in the legend to Fig. 3, except that the cultures were maintained at an A_{590} of between 0.1 and 0.5 by serial dilution. CH826 (recA441 e14⁰) cultures carrying either pRE-I (lanes 1 to 6) or pRE-X (lanes 7 to 12) were thermally induced at 42°C for 0 min (lanes 1 and 7), 70 min (lanes 2 and 8), 125 min (lanes 3 and 9), 185 min (lanes 4 and 10), 295 min (lanes 5 and 11), or 455 min (lanes 6 and 12).

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           HpaI        ←------ a ------→                          ---IHF-1
e14-----------GTTAACTGCTATTTTTATGCTAAAAATGGTTATCAAAACTCAAAAACACATGTTTATAATCAATGA
host----------GACGCCCTGGCAGCGCAGGTTGGCGGTATCGGTATCGCCCCTGGTGCAAACATCGGTGACGAATGC
              -150         .    -130         .    -110         .    -90


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GTTATAGAAATGCTAAGGGCTAATGAGTTATATGCAAATTAGTAAAATTATGTTGCTATGTCAGATAGTTACGATTTAGT
GCCCTGTTTGAAGCCACCCACGGTACTGCGCCGAAATATGCCGGTCAGGACAAAGTAAATCCTGGCTCTATTATTCTCTC
     .      -70         .    -50         .    -30         .    -10


     exchange                                        ----c----→
     _____  *            *         * *                          *
CATCTAACTAATGCTGCGCCACATGGGTTGGACCGAAGCGGCTGACTTAATTGTTAAAGGTATGGAAGGCGCAATCAACG
CGCTGAGATGATGCTGCGCCATATGGGTTGGACTGAAGCGGCTGACCTGATTGTTAAAGGTATGGAAGGCGCAATCAATG
              .    10         .    30         .    50         .    70
                NdeI


                          -----c----→
*  *      *          *         *        *                                  *
CGAAAACCGTAACCTATGACTTCGAGCGTCTGATGGATGGCGCTAAACTGCTGAAATGTTCAGAGTTTGGTGACGCGATC
CCAAGACCGTAACTTATGACTTCGAACGTCTGATGGAAGGCGCTAAGCTGCTGAAATGTTCAGAGTTTGGTGAAGCGATC
              .    90         .    110        .    130        .    150


                              ←-------- b ---------→
      **   **** *           *  ***      *  **    *  *
ATCGAAAACATGTAATGCCGTAGTTTGTTAAATTTATTAACGGGAGCGTAACGCTCCCGTTGTTTTTTGTTAGGCTGCTA
ATCGAAAACATGTAATCTCTCCATGTGTTAAATATTGAAACGGGCGTATAACACGCCCGTTGTTTTTATTTATGTGGATAT
              .    170        .    190        .    210        .    230


    ----IHF-2---

ACGGTTATCAAAATTTTATCAAAAAAAGTTATCAAAACCCCTCGGTAGTTTTG
TATTAATAGCATATCGAGCATATTTATATGAAGCCCATTACTTGGCCCATATG
              .    250        .    270        .
                                        NdeI
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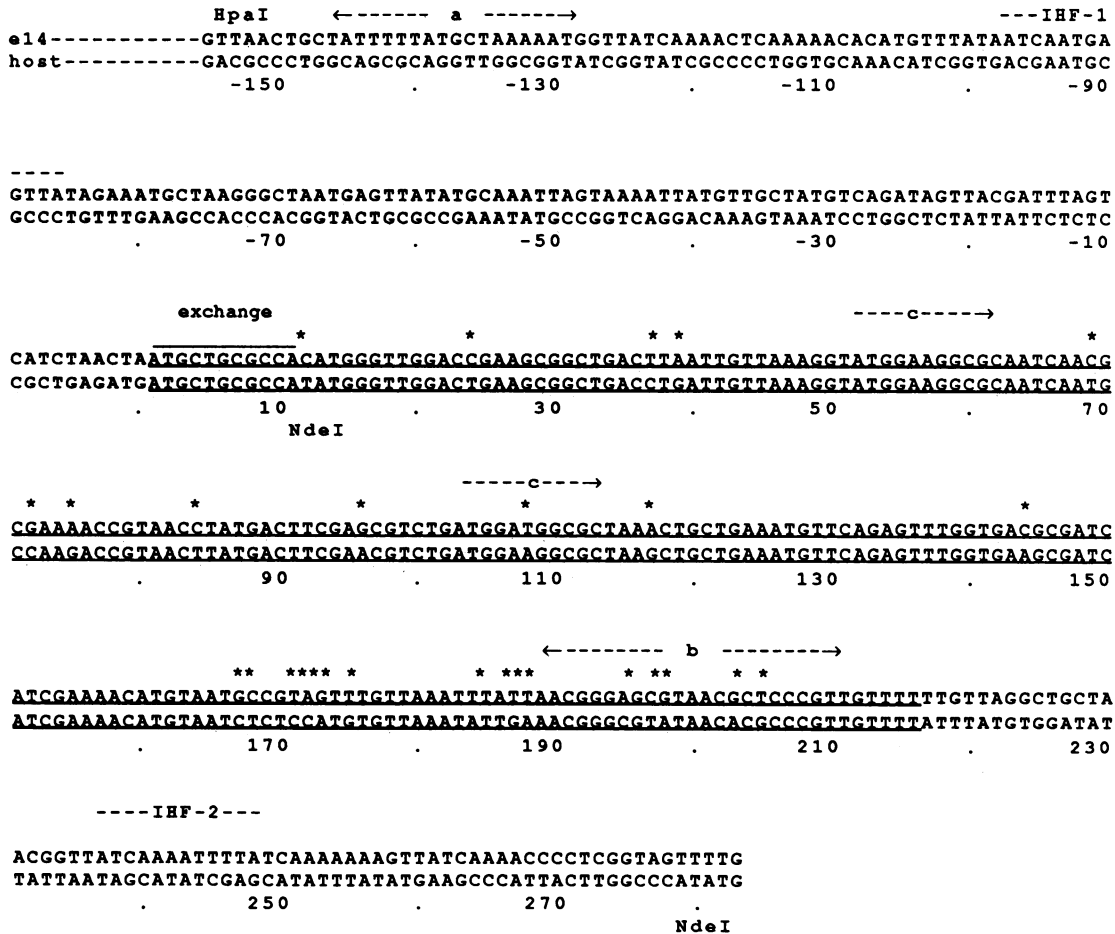
FIG. 5. Nucleotide sequences of the *attE* and *attC* regions. The upper sequence is *attE*, and the lower sequence is *attC*. The sequence shown for the *attE* region begins at the first position of the *Hpa*I site in the pHB108 insert, whereas the sequence shown for the *attC* region ends at the last position of a *Nde*I site which occurs approximately 670 bp to the left of a *Hpa*I site found in pHB206 (Fig. 1). The 216-base homology (coordinates 1 to 216) shared by *attE* and *attC* is underlined. Mismatches within the homology region are marked with an asterisk. The limits of the exchange region are indicated. Two sequences which match the IHF consensus in the *attE* region are indicated as IHF-1 and IHF-2. Other regions of direct or inverted repetition are indicated by lower-case letters.

observe significant amounts of supercoiled e14 after SOS induction of a K-12(e14+) culture (6). The lack of directionality was shown by the fact that regardless of whether pRE-I or pRE-X was present in the starting strain, a similar mixture of the two was found after 5 to 8 h. Apparently, the e14 site-specific recombination system works equally well in both directions once activated.

Our experiments show that the functional *attE* and *attC* sites clearly include the first 11 bp of the 216-bp homology (Fig. 5), since this was the site of the crossover. Beyond this, they do not define the limits of the attachment sites. Specifically, they do not indicate whether the entire homology is necessary or whether portions of the nonhomologous flanking regions are involved in the functional sites. If the functional attachment sites are strictly confined to the homologies, then *attE* is identical to *attL* and *attC* is identical to *attR*. If this were the case, then the lack of directionality (Fig. 4) would be expected.

Sites involved in site-specific recombination tend to exhibit a set of common features (10). For example, integration of temperate phages generally involves a core region that is homologous to the host attachment site. The 216-bp homology found for e14 is large when compared with the core

regions of 15 bp for lambda (9), 17 bp for φ80 (10), 20 bp for P4 (16), or 46 bp for P22 (10). Recently the attachment site of the SLP1 element of *Streptomyces coelicolor* was shown to have a 112-bp segment that was highly homologous to the host site (15). What role the homology region has in recombination is unclear. The actual crossover between e14 *attE* and *attC* occurred within the first 11 bp of the homology. Therefore, by analogy with the temperate phages, it might be appropriate to consider only this 11 bp to be the e14 attachment core. The remaining 205 bp of homology may have some other significance. It has been proposed that in P22, much of the homology between the bacteriophage and the chromosomal attachment sites was the result of an imprecise excision of a P22-type prophage at some earlier stage in the divergence of the host strain (10). A similar explanation has been offered to account for P4 related sequences that occur adjacent to the P4 attachment site in *E. coli* K-12, but not in *E. coli* C (16). An analogous derivation of the host homology region is a possibility for e14. *E. coli* B, *E. coli* C, and cured *E. coli* K-12 all contain a large *Hind*III fragment that is conserved in size and extent of hybridization to an *attE*-specific probe (4). For strains K-12 and C, this fragment contains the functional host attachment site (strain

B was not tested). The strength of the hybridization of the fragments from the B and C strains was very similar to that of K-12 (e14°), suggesting comparable homology to e14. Therefore if a major part of the homology was due to a remnant fragment from a related prophage, the event would seem to have occurred early, before the divergence of these strains.

Another feature commonly shared by sites involved in site-specific recombination is the presence of a region of twofold rotational symmetry. The *attE* sequence contains an 11-bp inverted repeat near the right end of the 216-bp homology region (designated b in Fig. 5), whereas *attC* contains a related 9-bp inverted repeat at the same position. An additional common feature of sites involved in site-specific recombination is the presence of integration host factor (IHF) binding sites. These were expected in the e14 system because e14 excision after thermal induction of SOS is reduced to undetectable levels by a *himA* mutation in IHF (A. Greener, Ph.D. thesis, Pennsylvania State University, University Park, 1981). In fact, two perfect matches to the IHF consensus Pyr-AANNNNTTGATT/A (10) were found near *attE*. Their reverse complements are designated IHF-1 and IHF-2 in Fig. 5. IHF-2 begins 20 bp to the right of the homology, and IHF-1 begins 80 bp to the left. Furthermore, IHF-2 is 36 bp from the center of the 11-bp dyad b. This is similar to arrangements in lambda, φ80, and P22, in which IHF binding sites and dyad symmetries are separated by 32 to 37 bp. In addition, this putative e14 IHF site has the same orientation toward the dyad as is observed for these temperate phages (10). The analogy between e14 and these temperate phages breaks down, however, in that the crossover for e14 did not occur within the dyad symmetry. Instead, it occurred roughly 200 bp away at the other end of the homology. The other potential IHF binding site lies 36 bp from a very A+T-rich 7-bp dyad symmetry designated a in Fig. 5.

Classification of e14 among accessory genetic elements remains problematic. Its apparent use of core homology for site-specific recombination is more similar to the temperate phages than to the transposable elements. As noted above, aspects of the organization of dyad symmetries and potential IHF binding sites are similar to those of lambdoid temperate phages, but the position of the strand exchange relative to these sites is extremely atypical. The lack of directionality of the system also contrasts to that expected for temperate phages such as lambda, which have a highly developed and controlled integration-excision system that ensures efficient integration for the lysogenic response and efficient excision for lytic induction (5). It is quite possible that e14 is not a prophage at all, but belongs to some other class of element such as the plasmidogenic class proposed by Omer and Cohen (15) to explain the properties of the SLP1 element of *S. coelicolor*. Control of the directionality of recombination might be less important to an element that alternates between a plasmid and a chromosomal state.

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