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Sequence Analysis and Comparison of *int* and *xis* Genes from Staphylococcal Bacteriophages L54a and φ11

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The DNA fragment encoding the integrase and excisionase genes involved in site-specific recombination of staphylococcal bacteriophage φ11 was cloned and sequenced. The *int* and *xis* genes and the recombination site, *attP*, were highly clustered in a 1.7-kilobase DNA fragment with the gene order *attP-int-xis*. The *int* and *xis* genes were transcribed divergently, with the *int* gene transcribed toward the *attp* site and the *xis* gene transcribed away from the *attP* site. The deduced Int is a basic protein of 348 residues with an estimated molecular weight of 41,357. In contrast, the deduced Xis is an acidic protein containing 66 amino acids with an estimated molecular weight of 7,621. The site-specific recombination system of φ11 was compared with that of a closely related bacteriophage, L54a.

Staphylococcal bacteriophage φ11 is a group B temperate bacteriophage originally isolated from *Staphylococcus aureus* 8325 (27). It is the best genetically characterized among all staphylococcal bacteriophages. The φ11 DNA is a linear, double-stranded, circularly permuted, and terminally redundant molecule of about 45 kilobases (kb) (1, 4, 23, 24). The extent of terminal repetition is about 5% of the genome length (25). The circular genetic and restriction maps have been established (2, 17, 25). As a temperate bacteriophage, φ11 is capable of both lytic and lysogenic life cycles. During lysogeny, the viral DNA recombines with the host chromosome between the viral attachment site (*attP*) and the bacterial attachment site (*attB*). Integration generates two additional sites, *attR* and *attL*, at the right and left junctions between the prophage and the host chromosome, respectively. The *attB* site is located between the *purB* and *metA* loci (30). Recently, DNA sequencing revealed that all four *att* sites shared a 10-base-pair (bp) common core sequence (21).

We have previously studied the site-specific recombination system of another staphylococcal bacteriophage, L54a (18, 21, 37). φ11 and L54a are closely related phages; they belong to the same serogroup B and lytic group III, they are homoimmune, and their DNAs cross-hybridize extensively (25; unpublished data). Integration and excision of L54a follow the same model as that of φ11. The *att* sites of L54a also share a short (18-bp) core sequence. The core sequences of the attachment sites of both phages have 6 bp of nucleotide homology. In addition, an 11-bp direct repeat with four repetitions in the φ11 *attP* site is arranged similarly to that of a 12-bp direct repeat in the L54a *attP* site (20, 21). Thus, the site-specific recombination systems of both phages may be machanistically similar. However, the two phages differ in their bacterial attachment sites. To compare the two systems at the molecular level and to begin to understand the molecular nature of the specificity, we cloned and sequenced two φ11 recombination genes, *int* and *xis*, which are located adjacent to the *attP* site. We have previously isolated, mapped, and sequenced the recombination genes of L54a (20, 37). Here we compare the site-specific recombination system of φ11 with that of L54a.

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MATERIALS AND METHODS

Bacterial strains and phages. *S. aureus* RN4220, a restriction-deficient strain derived from *S. aureus* 8325-4 (16), was used as the recipient in transformations as well as the host for plasmid integrations. Protoplast transformation was carried out by the procedure of Chang and Cohen (5). *Escherichia coli* LE392 was used for plasmid transformations and for preparation of plasmid and cloned DNA. Plasmid DNA purification was performed by the procedure of Birnboim (3) and further purified by CsCl-ethidium bromide density gradient centrifugation. Phage φ11 DNA was isolated as previously described (19). Bulk chromosomal DNA from *S. aureus* was purified by the method of Dyer and Iandolo (8).

Chemicals and enzymes. Trypticase soy broth (Difco Laboratories, Detroit, Mich.) was used for routine cultivation of *S. aureus* strains. L broth (Difco) was used for cultivation of *E. coli* cells. Chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). Restriction enzymes, BAL 31 exonuclease, bacteriophage T4 DNA ligase, exonuclease III, and nick translation reagents were obtained from New England BioLabs, Inc. (Beverly, Mass.), and Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). The large fragment of DNA polymerase (Klenow fragment) and DNA sequencing kit including sequenase were obtained from U.S. Biochemical Corp. (Cleveland, Ohio). [^{32}P]dCTP and [^{35}S]dATP were purchased from New England Nuclear Corp. (Boston, Mass.). The penicillinase (BlaZ) assay was performed essentially as described previously (30, 31). To detect a small number of BlaZ$^-$ colonies in a large population of BlaZ$^+$ colonies, plates containing methicillin (Sigma) and starch with up to 10^5 colonies were incubated at 37°C for about 10 h and flooded with solution containing penicillin G (Sigma), iodine, and potassium iodide (31). BlaZ$^-$ colonies were then detected by using a stage microscope or a low-power microscope. Under such conditions, BlaZ$^-$ colonies appeared dark in a light background.

Recombinant DNA methods. General DNA manipulations were performed as described by Maniatis et al. (26). Rapid small-scale DNA purification was done by the method of Holmes and Quigley (14). The transfer of DNA to nitrocellulose membranes was done by the method of Southern (34). The hybridization conditions were as previously described (19).

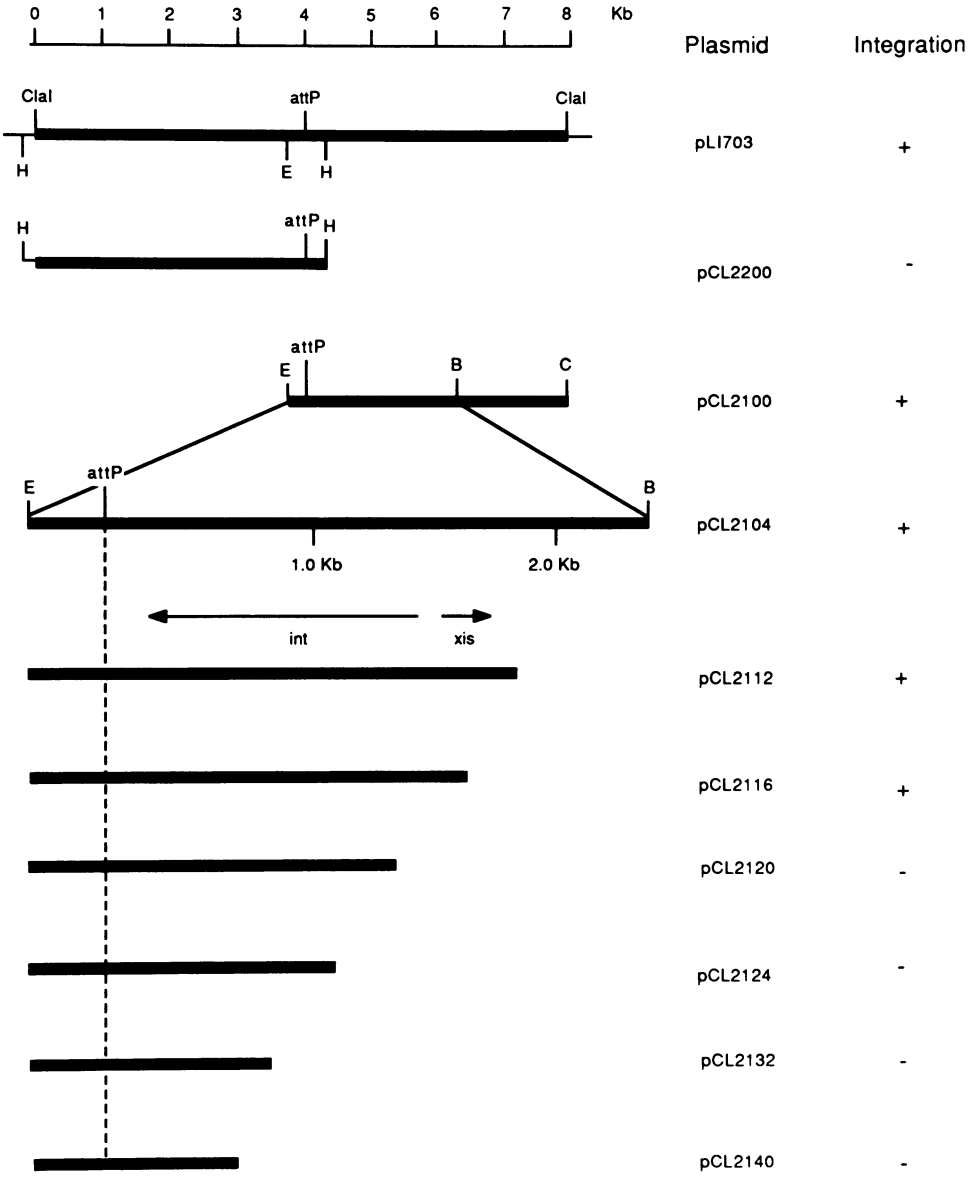

FIG. 1. Localization of the *int* gene near the *attP* site. The dotted line indicates the approximate location of the core sequence within the *attP* site. The intregration activity of each deleted plasmid, as determined by the Southern hybridization shown in Fig. 2, is indicated. Arrows indicate the *int* and *xis* structural genes. Abbreviations: E, *Eco*RI; H, *Hin*dIII; B, *Bgl*II; C, *Cla*I.

DNA sequence analysis. The unidirectional exonuclease III deletion procedure (11, 13) was used to generate overlapping deletions of the DNA fragment for sequencing. The 1.8-kb insert from pCL2112 was cloned into bacteriophage M13 derivatives mp18 and mp19 (36). The DNA was double digested with appropriate restriction endonucleases in the polylinker region of the vector between the insert and the sequencing primer-binding site to generate a 5′ protruding end or a blunt end adjacent to the insert and a 3′ protrusion adjacent to the sequencing primer-binding site. Exonuclease III was added to the linearized DNA. Samples were removed at various times, treated with S1 nuclease and Klenow fragment, and then ligated with T4 DNA ligase. The ligated mixtures were transformed to competent *E. coli* JM103. The single-stranded DNA was isolated for sequencing by the dideoxy-chain termination method of Sanger et al. (32). The entire DNA fragment was sequenced on both strands.

RESULTS

Cloning of the ϕ11 *int* and *xis* genes. Since in most reported systems of phage site-specific recombination, the *attP* site is tightly clustered with the *int* and *xis* genes, our initial attempt to clone the *int* and *xis* gene of ϕ11 was to clone the DNA fragment near the *attP* site. The ϕ11 *attP* site has been mapped in the circular ϕ11 map (25). Previously, we cloned an 8-kb *Cla*I fragment containing the *attP* site of ϕ11 in plasmid pBR322 (21). This plasmid, pLI703, was digested with *Hin*dIII, and the 4.5-kb *Hin*dIII fragment was cloned into the *Hin*dIII site of pLI50 (an *S. aureus-E. coli* shuttle vector) to generate plasmid pCL2200. A 4.2-kb *Eco*RI-*Cla*I fragment of pLI703 was also isolated and cloned into pLI50 to generate plasmid pCL2100. Thus, plasmids pCL2200 and pCL2100 contained the ϕ11 *attP* site with DNA extending leftward and rightward from it, respectively (Fig. 1). The

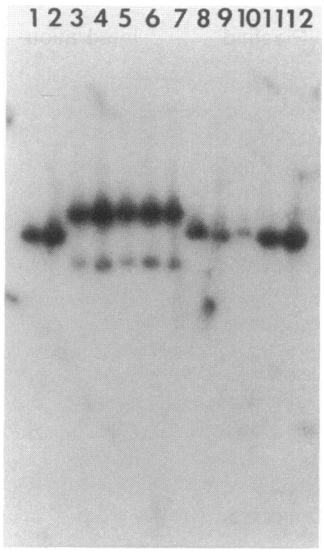

FIG. 2. Southern hybridization analysis to determine integration. The DNA from transformants was digested with *Cla*I, subjected to electrophoresis in agarose gel, and blotted onto nitrocellulose papers. The blots were hybridized with a radioactive probe prepared from the *Hin*dIII fragment containing the *attB* site. Digested DNA was from the following (lanes): 1, RN4220; 2, RN4220(pCL2200); 3, RN4220(pCL2100); 4, RN4220(pCL2104); 5, RN4220(pCL2112); 6, RN4220(pCL2116); 7, RN4220(pCL2118); 8, RN4220(pCL2120); 9, RN4220(pCL2124); 10, RN4220(pCL2128); 11, RN4220(pCL2132); 12, RN4220(pCL2140).

plasmids were transformed into protoplasts derived from *S. aureus* RN4220, and the presence of recombinase activity was tested by Southern blotting (34) as follows. Bulk chromosomal DNA was prepared from the transformants and digested with restriction enzyme *Hin*dIII. The digested DNA was hybridized to a probe of the 1.4-kb *Cla*I fragment containing the *attB* site from the chromosome of strain 8325-4 (21). Since *Hin*dIII cleaves plasmids pCL2100 and pCL2200 at least once but does not cleave the 1.4-kb *Cla*I fragment, the probe would identify only one band if no integration occurred, whereas it would identify two bands if the plasmids had integrated. Plasmid pCL2200 was unable to integrate, whereas plasmid pCL2100 was able to integrate (Fig. 1), indicating that the *int* gene was located within the 4.2-kb DNA segment rightward to the *attP* site.

The DNA fragment between the *Bgl*II and *Cla*I sites of pCL2100 was then deleted by restriction endonuclease digestion and religated by T4 DNA ligase. The resultant plasmid was able to integrate, as revealed by Southern analysis (Fig. 2), indicating that the φ11 *int* gene is located within a 2-kb fragment between the *attP* site and BglII site (Fig. 1). Further mapping of the *int* gene was done by deleting DNA from the *Bgl*II site toward the *attP* site by BAL 31 deletion. Plasmid pCL2100 was linearized by *Bgl*II digestion and treated with exonuclease BAL 31 for various lengths of time. The ends of the linear plasmids were made flush with the Klenow fragment and religated with T4 DNA ligase. The resultant plasmids with deletions of different lengths were first propagated into *E. coli* LE392 and characterized and then transformed into protoplasts derived from strain RN4220. The integration activity was tested by Southern blotting as described above by using the 1.4-kb *Cla*I fragment containing the *att*φ11 site as a probe (Fig. 2). The results summarized schematically in Fig. 1 indicated that the *int* gene was located within a 1.55-kb DNA segment rightward from the *attP* site (in plasmid pCL2116). It is likely that the promoter of the *int* gene is also located within the 1.55-kb DNA fragment. This is also supported by the observation that the recloning of the 1.55-kb fragment into another vector resulted in *int* expression (data not shown). However, we cannot rule out the possibility that the transcription started from a promoter carried in the vector.

Sequence analysis of the *int* and *xis* genes. The results in Fig. 1 indicate that plasmid pCL2116 carried the intact *int* gene. To sequence the DNA fragment containing both the *int* and *xis* genes, we chose the φ11 DNA fragment carried on pCL2112 for sequencing because this fragment is likely to contain both complete genes. This is based on the assumption that the site-specific recombination system of φ11 is similar to that of L54a, a staphylococcal temperate bacteriophage closely related to φ11, in which *attP*, *int*, and *xis* are highly clustered within a 1.75-kb DNA fragment (37). The 1.8-kb fragment from pCL2112 was recloned to bacteriophage M13 vectors M13mp18 and M13mp19. The entire fragment was sequenced on both strands as described in Materials and Methods. The complete nucleotide sequence containing the *int* and *xis* genes and the deduced amino acid sequences is shown in Fig. 3. We found two open reading frames (ORFs) capable of encoding proteins longer than 50 residues (although we also found several small ORFs, they were less than 46 residues long and none was preceded by a potential ribosome-binding site). The longer ORF was translated rightward toward the *attP* site from position +1134 extending to position +91 and was capable of encoding a protein with 348 amino acids. This ORF was identified as the coding region for φ11 Int protein, because plasmids with deletions into this reading frame were unable to integrate to the *attB* site (Fig. 1). The *int* start codon is most likely at position +1134, because this is the only start codon that is preceded with a potential ribosome-binding site (33) and because plasmid pCL2120, with a deletion of sequences just beyond this start site, was unable to integrate.

The smaller ORF was translated right to left from positions +1246 to +1444 and was capable of encoding a protein with 66 residues. This ORF is likely to be the coding region for Xis protein for the following reasons: (i) the size of the deduced protein is similar to those of L54a Xis and lambda Xis, (ii) and pI value of the deduced protein is very close to that of L54a Xis, and (iii) the relative location and the direction of transcription with respect to *int* and *attP* are the same as those of L54a. To further confirm that this smaller ORF encoded the Xis protein, we constructed plasmid pYL124 and pYL125 as described in the legend to Fig. 4. Plasmid pYL124 contained an intact putative *xis* ORF, whereas pYL125 contained an approximately 20-bp deletion at the 3′ end of the ORF. Both plasmids contained the intact *int* ORF and the *attP* site. In addition, both plasmids carry the *blaZ* gene from plasmid pI258 (28) and the tetracycline resistance gene from pT181. These two plasmids were transformed into *S. aureus* RN4220 with selection for tetracycline (3 μg/ml) resistance. Because the plasmids contained no replication function to replicate in *S. aureus*, they integrated into the *attB* site on the chromosome. The resultant strains, RN4220(pYL124) and RN4220(pYL125), were BlaZ$^+$ by the plate detection method (30, 31). These strains were used to test for excisive ability by assaying for BlaZ activity. Because an integrated plasmid that carries a functional *xis* gene can excise at a rate similar to phage excision, conversion of BlaZ can be detected in a strain containing the integrated plasmid at a detectable level. On the other hand, an inte-

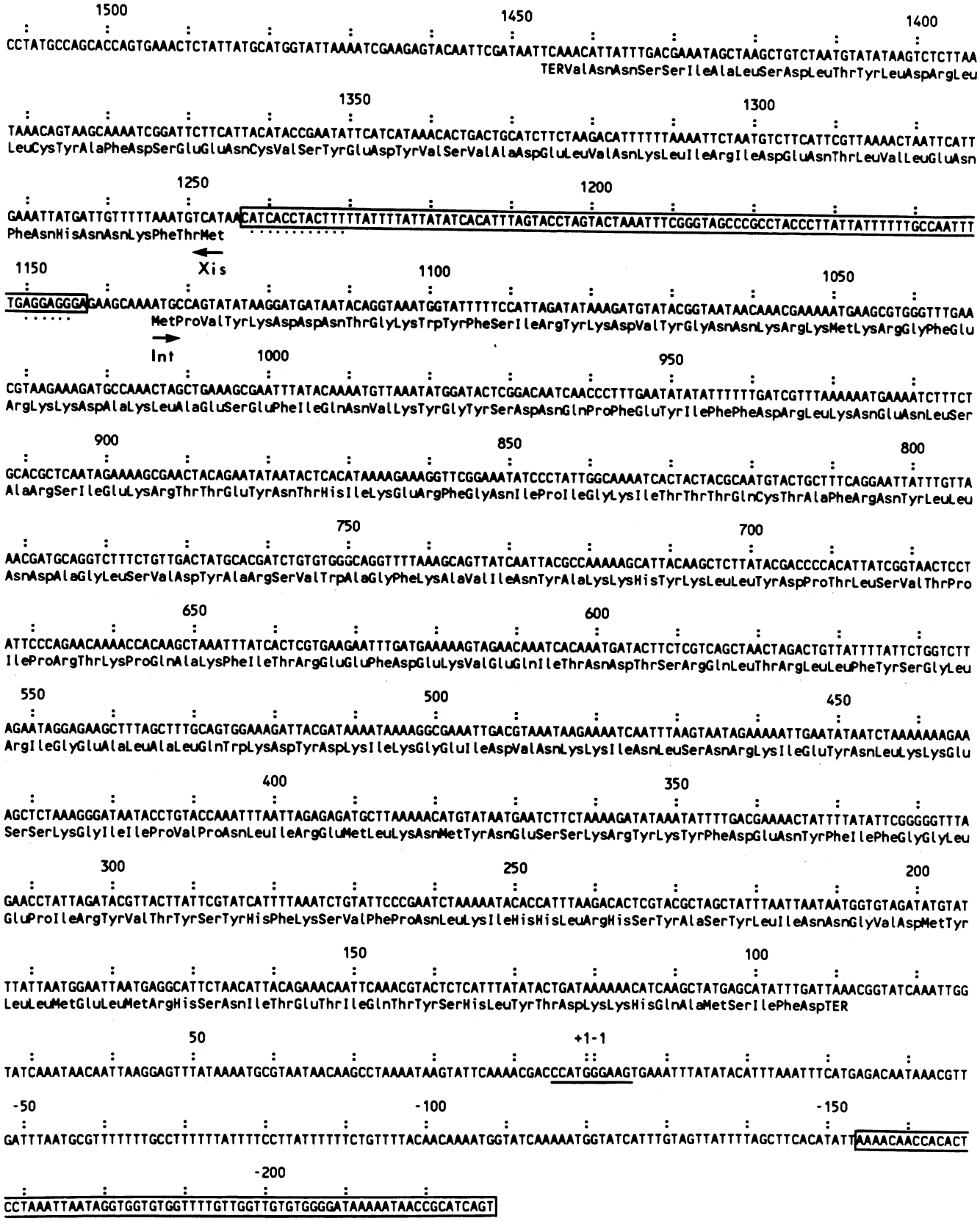

FIG. 3. Nucleotide sequence and predicted amino acid sequence of the *attP-int-xis* region of ϕ11. Sequence is numbered from the center of the core; the base immediately to the left is designated as +1, and the base immediately to the right is designated as −1. The possible Shine-Delgarno sequences are dotted underneath. Termination codons are designated by TER. The complete core sequence of the *attP* site is underlined. Arrows indicate the start sites and orientations of the ORFs for *int* and *xis* genes. The nucleotide sequences in the boxes indicate the highly homologous regions of L54a and ϕ11.

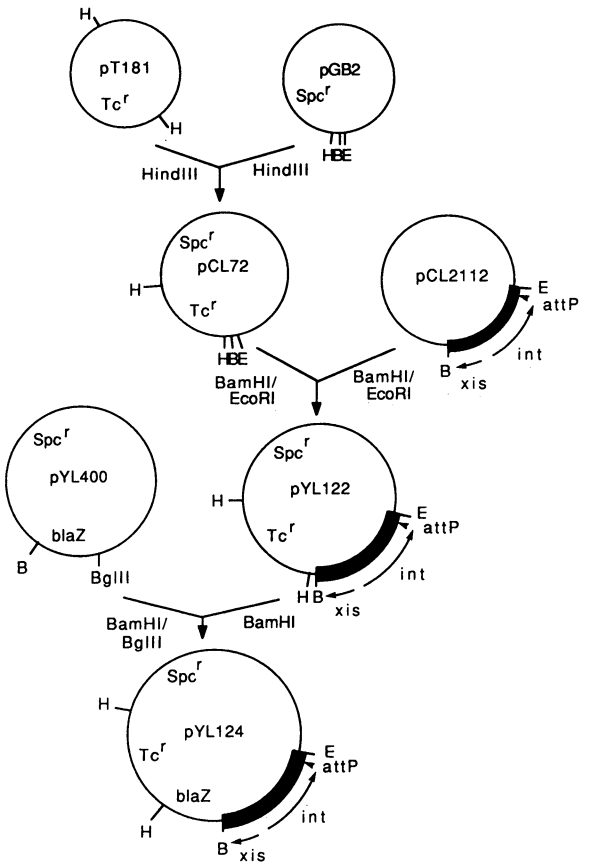

FIG. 4. Construction of plasmids pYL124 and pYL125. Plasmid pT181 was digested with *Hin*dIII, and the 2.4-kb fragment containing the tetracycline resistance gene (15) was cloned into the *Hin*dIII site of pGB2 (6). The resultant plasmid, pCL72, was digested with *Eco*RI and *Bam*HI and ligated with the 1.8-kb *Eco*RI-*Bam*HI fragment containing the *attP* site, the *int* gene, and the putative *xis* gene from pCL2112. The resultant plasmid, pYL122, was digested with *Bam*HI and ligated with the 1.1-kb *Bam*HI-*Bgl*II fragment containing the *blaZ* gene from pYL400 to generate plasmid pYL124. Plasmid pYL400 was constructed by ligating the 1.1-kb *Hin*dIII-*Taq*I fragment containing the *blaZ* gene from plasmid pI258 (28, 35) to the *Hin*dIII and *Acc*I sites of plasmid pGB2. Plasmid pYL125 was constructed the same way as pYL124, except a 20-bp sequence was deleted at the 3′ end of the *xis* ORF (i.e., the *attP-int-xis* region was derived from pCL2126). Abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII.

grated plasmid that does not carry the functional *xis* gene cannot excise, and no conversion can be detected in a strain containing the plasmid. Strain RN4220(pYL124) reverted from BlaZ$^+$ to BlaZ$^-$ at a rate of about 10^{-4}, whereas strain RN4220(pYL125) showed no reversion ($<10^{-4}$). This result implies that the smaller ORF that is intact in pYL124 and partially deleted in pYL125 encodes for Xis protein.

In Fig. 3, a potential ribosome-binding site was also found preceding the *xis* ORF. The *int* and *xis* genes were transcribed divergently with only 111 bp in between the start sites of the two genes. We did not find putative promoter consensus sequences like those of *E. coli* (10) immediately upstream from either ORF.

Features of Int and Xis amino acid sequences. Computer analyses predicted that the φ11 *int* gene product was a 348-residue protein with an calculated molecular weight of 41,357 (Fig. 3). The size of the protein was similar to those of the 356-residue lambda Int and the 354-residue L54a Int (7, 12, 37). Like most DNA-binding proteins, φ11 Int was highly basic with 67 basic (19.3%) and 40 acidic (11.5%) residues. The estimated pI was about 10.5. A region of the protein near the N terminus between residues 26 and 40 contained a high proportion of basic residues (9 of the 15 residues are basic). The predicted φ11 Xis protein contains 66 amino acids with an estimated molecular weight of 7,621. The protein is a small acidic protein with 5 basic and 12 acidic residues. The pI value of the φ11 Xis protein is about 5.0. The acidic nature is similar to L54a Xis but distinct from the basic Xis proteins of lambda, P22 and φ80 (7, 12, 22, 37).

Substrate specificity of integrase. Although L54a and φ11 are closely related phages, the *attB* sites for L54a and φ11 are different. The difference in integration site on the bacterial chromosome suggests that the two recombinases differ in specificity. However, it is also possible that each of the integrases can promote recombination of a pair of attachment sites of other phage. To test this possibility, a plasmid containing φ11 *int* but not *attP* was constructed by deleting the φ11 *attP* site from pCL2116 (Fig. 1) with BAL 31. The resultant plasmid, pCL2116-8, was transformed into strain RN4220(pYL101) (37), and the lipase activity of the transformants was tested. Because pYL101, which is compatible with pCL2116-8, contains the L54a *attP* site, it would integrate into the *att*L54a site and cause a lipase-negative phenotype of the transformants if the φ11 Int promoted integration of L54a. The transformants remained lipase positive (data not shown), indicating that φ11 Int cannot promote recombination of L54a *attB* and *attP* sites. As a control, plasmid pCL2116-8 was also transformed into RN4220 containing a compatible plasmid carrying the φ11 *attP* site but not the *int* gene. Southern hybridization confirmed that pCL2116-8 contained functional Int (data not shown). Using similar experimental design, we also showed that L54a Int could not promote recombination of φ11 *att* sites (data not shown).

DISCUSSION

We cloned and sequenced the φ11 DNA fragment containing the *int* and *xis* genes required for site-specific recombination of φ11. The *int* gene was identified by correlating the results of deletion mutagenesis to the changes of integrative phenotype determined by Southern hybridization. Due to the lack of a suitable assaying system, the *xis* gene was first identified by directly comparing the ORF deduced from the nucleotide sequence of φ11 with that of L54a. The ORF for *xis* gene was then confirmed by correlating the ability of the putative *xis* ORF to excise an integrated plasmid carrying a *blaZ* reporter gene.

Our previous structural analyses of the attachment sites of φ11 and L54a indicated that the site-specific recombination pathways of the two phages may share similar mechanistic features. Furthermore, the core sequences of their attachment sites have 6 bp of homology (20, 21). However, when the Int proteins of φ11 and L54a were compared, we found no global homology but only limited local homologies at the C-terminal halves (Fig. 5). One homologous region of about 40 residues near the C-terminal end was also found in 15 other recombinases belonging to the integrase family (1, 29). This region of homology includes three perfectly conserved residues in which the active sites of 2 of the 15 recombinases were found to be at one of the perfectly conserved residues, tyrosine. In view of the fact that φ11 Int also contains the three perfectly conserved residues at the same relative

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L54a                MFRLEEKIKEKLNNKSSSELKTLTFHALLDEWLEYHIKTSGFKVTTLD 49
 φ11  MPVYKDDNTGKWYFSIRYKDVYGNNKRKMKRGFERKKDAKLAESEFIQNVKYGYSDNQPFE 61

L54a  NLKTRIKNIKKNSSQNLLLNKIDTKYMQTFINELSNVYSANQVKRQLGHMKEAIKYAVKFY 109
 φ11  YIFFDRLKNENLSARSIEKRTTEYNTHIKERFGNIPIGKITTTQCTAFRNYLLNDAGLSVD 122

L54a  NYPNEHILNSVTLPKKSKTIEDIEKEEAKMYNYLEMEQVIQIRDFILNDNNMQYRARILVA 170
 φ11  YARSVWAGFKAVINYAKKHYKLLYDPTLSVTPIPRTKPQAKFITREEFDEKVEQITNDTSR 183

L54a  GAVEVQALTGMRIGELLALQVKDVDLKNKTIAINGTIHRIKCNAGFGHKDTTKTAGSKRKI 232
 φ11  QLTRLLFYSGLRIGEALALQWKDYDKIKGEIDVNKKINLSNRKIEY----NLKKESSKGII 240

L54a  AINSRIANVLKKIMLENKKMQQWEPSYVDRGFIFTTCQGNPMQGSRINKRLSSAAESLNIN 293
 φ11  PVPNLIREMLKNMYNESSKRYKY---FDENYFIFGG--LEPIRYVTYSYHFKSVFPNLKI- 295

L54a  KKVTTHTLRHTHISLLAEMNISLKAIMKRVGHRDEKTTIKVYTHV-TEKMDRELEQKLEKLVY 354
 φ11  -----HHLRHSVASYLINNGVDMYLLMELMRHSNITETIQTYSHLYTDKKHQAMSIFD     348

Int       H-LRHT-AS-L---G-----IQ--LGH---------Y
Family    * *                   *
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FIG. 5. Comparison of the Int proteins of L54a and φ11. The two proteins were compared by using the FASTP algorithm of Lipman and Pearson (23). A colon indicates identical amino acids. A dot indicates amino acids in the same exchange groups. Relatively significant homology was found in the C-terminal region of the two integrases. The conserved amino acids of the Int family are also aligned with the L54a and φ11 Int proteins. The three perfectly conserved residues of the Int family are indicated by asterisks. The identical residues of the L54a and φ11 Int proteins to the conserved residues of the Int family are underlined. Data for the Int family are from Argos et al. (1) and Pargellis et al. (29).

positions, we suggest that the active site of φ11 Int is also at Tyr-332 (Fig. 5). The lack of homology may reflect specificity, since we showed that neither integrase complemented recombination of the other phage. However, the degree of divergence between the proteins is intriguing. This is not surprising, because a similar degree of divergence of the Int proteins among lambda-related phages was previously reported (22).

The Xis proteins of φ11 and L54a have no homologous amino acid sequences; however, they are about the same size and are both acidic. The acidic nature is different from the basic nature of the Xis proteins of lambda, P22, and φ80 (7, 12, 22), though all of these Xis proteins are both the same size. The difference in acidity suggests that the mechanism of excisive recombination of φ11 and L54a may be different from that of lambdoid phages.

As in lambda, P22, φ80, and L54a, the genetic functions involved in site-specific recombination of φ11 are highly clustered within a short stretch of DNA fragment with the gene order *attP-int-xis* (7, 12, 22, 37). The *int* and *xis* genes of φ11 are transcribed divergently, which is similar to transcription of L54a. In contrast, the genes of lambda and P22 are transcribed in the same direction and the genes of φ80 are transcribed convergently. This finding indicates that the regulation of the expression of *int* and *xis* genes of φ11 is likely similar to that of L54a but different from those of lambda, P22, and φ80.

When we compared the nucleotide sequence of the DNA fragment containing the *attP-int-xis* region of φ11 with that of L54a (37), we did not find significant homology between the *int* coding regions, the *xis* coding regions, or the *attP* sites of the two phages. However, a high degree of homology (97%) was found at the region between the translation start sites of *int* and *xis* genes (Fig. 6). This homology is remarkable, given the fact that the sequences on either sides of this region had no homology between the two phages. The existence of this highly conserved region indicates that the sequences in this region may be functionally important. Because this region is located just upstream from the start sites of both genes, it is likely that the potential regulatory

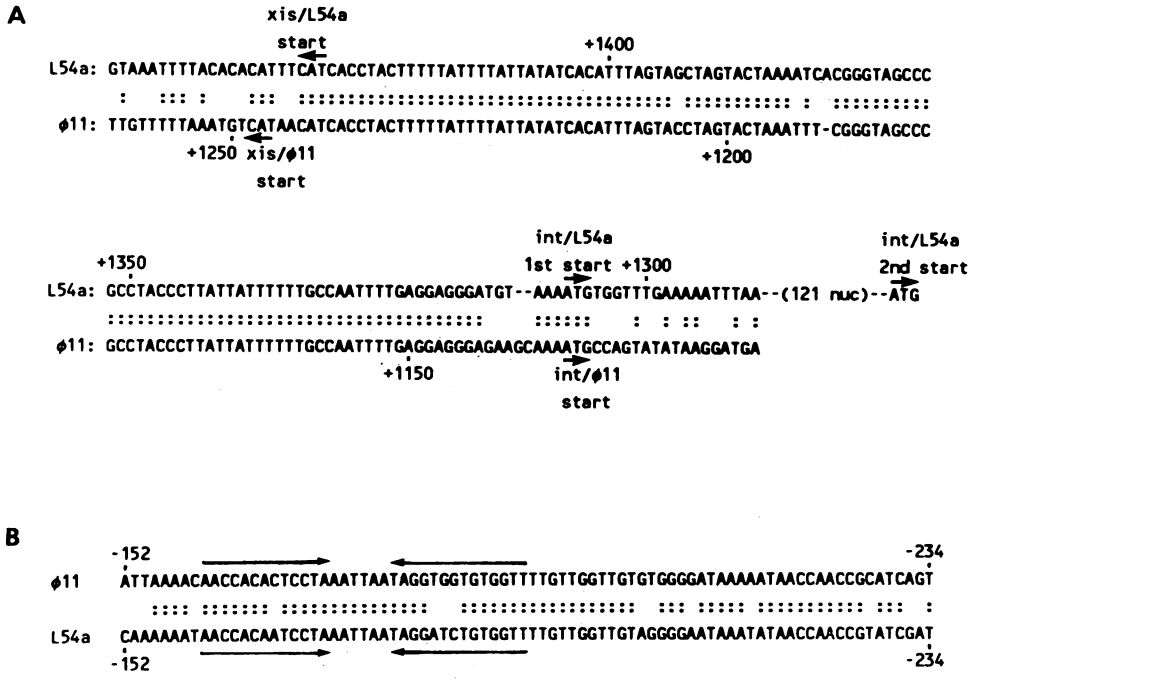

FIG. 6. Comparison of the nucleotide sequences of the *attP-int-xis* region of φ11 and L54a showing the conserved regions. Numbering of the bases is the same as shown in Fig. 3. Colons between nucleotides indicate identity. (A) Sequences at the region between the translation start sites of *int* and *xis* genes. The translation start sites and the direction are shown by arrows. (B) Sequences at the region downstream of the *int* gene beyond the *attP* site. Inverted arrows indicate the inverted repeat sequences.

sites for the expression of the *int* or *xis* gene are located within this region. However, we did not find putative promoter sequences preceding either gene at this region in either phage ϕ11 or L54a. The highly homologous region also suggests that the regulatory mechanisms of the expression of *int* and *xis* of ϕ11 are very similar to those of L54a. However, we previously showed that the L54a Int translated from the second start codon 142 bp downstream from the first start codon is functional (37). Thus, the regulation of *int* between ϕ11 and L54a may be different to some extent.

The other region with high homology to phage L54a is the region downstream from the *int* gene just beyond the *attP* site (i.e., the region at the opposite side of the *int-xis* coding region with respect to the *attP* site; Fig. 6). From the available sequencing data we found 85% sequence homology at this region. In addition, we also found an almost perfect 13-bp inverted repeat in this region. In lambda, an *sib* site with an inverted repeat negatively regulates the *int* expression (termed retroregulation) and is located at the same relative location (for a review, see reference 9). Whether this region has any role in regulating the *int* expression in ϕ11 or L54a requires further investigations. Phages ϕ11 and L54a are closely related phages. The finding of two regions of nucleotide sequence homology between the two phages further lends support to the notion that these two phages evolved from a common ancestor.

In summary, we cloned and sequenced the *int* and *xis* genes of ϕ11 and showed that the genetic organization of the site-specific recombination functions (*attP*, *int*, and *xis*), of ϕ11 is very similar to that of L54a. Although we found no global homology either at the DNA or protein level between the Int genes and between the Xis genes of ϕ11 and L54a, significant nucleotide sequence homologies were found at the potential regulatory regions.

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