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Structural Analysis of Staphylococcal Bacteriophage φ11 Attachment Sites

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The lysogenization of bacteriophage φ11 in *Staphylococcus aureus* occurs by site-specific recombination. The DNA segments containing the attachment sites on the host chromosome, the phage genome, and the two junctions created by insertion of the prophage were cloned, and the nucleotide sequences were determined. The attachment sites share a very short common sequence of 10 base pairs.

Bacteriophage φ11 is a group B phage carried as a prophage in *Staphylococcus aureus* NCTC 8325 (9). Genetically, it is the most characterized of all staphylococcal phages. The viral DNA is a linear, double-stranded, terminally redundant molecule of about 45 kilobases (kb) (1, 6, 7). In addition to the viral attachment site and the immunity region, 10 genes have been located on a circularly permuted map (3, 6). During lysogeny, insertion into the chromosome occurs by site-specific recombination between the viral attachment site (*attP*) and the *att*φ11 site (12) on the host chromosome. To define the attachment site on the viral genome more precisely and to study the site-specific recombination mechanism, we cloned and determined the nucleotide sequences of (i) the attachment site on the phage genome (*attP*), (ii) the attachment site on the bacterial chromosome (*attB*), and (iii) the attachment sites generated at the junctions between φ11 and *S. aureus* DNA (*attR* and *attL*).

Phage φ11 DNA and bulk chromosomal DNA were isolated as previously described (2). We identified the fragments containing *attR*, *attL*, and *attP* directly by hybridization. Chromosomal DNA from φ11-lysogenized strain 8325-4 and φ11 DNA were digested with the restriction endonuclease *Cla*I. The fragments were separated by agarose gel electrophoresis on adjacent gel lanes and transferred to nitrocellulose by Southern blotting (14). The blot was probed with ^{32}P-labeled φ11 DNA, and the profiles were compared. Because integration occurs between *attB* and *attP* sites and because the genome of φ11 is circularly permuted, φ11 gene probes should identify two unique bands in the genomic DNA of the lysogenized strain and one unique band in the genomic DNA of phage φ11. Two unique junction fragments that contained *attL* (on a 5.1-kb fragment) and *attR* (on a 4.3-kb fragment) were identified. One unique fragment (8 kb) from the φ11 digest that contained *attP* was identified. These fragments were isolated and cloned into the *Cla*I site of pBR322.

To identify the DNA fragment containing the *attB* site, we digested bulk chromosomal DNA from strain 8325-4 (a nonlysogenized derivative of NCTC 8325) with *Cla*I, electrophoresed the digest on an agarose gel, and then transferred it to nitrocellulose. A ^{32}P-labeled DNA fragment containing either *attR* or *attL* was used for blot hybridization because such a probe was partially homologous to the DNA fragment carrying *attB*. A 1.4-kb *Cla*I fragment that contained *attB* was identified. The fragment was isolated and cloned into the *Cla*I site of pBR322.

The primary DNA fragments isolated were subjected to further restriction mapping to identify the smaller (secondary) fragments containing the *att* sites. The logic used to identify the secondary fragments was the same as that of Landy and Ross (4). The primary fragments containing the four *att* sites, *attP*, *attL*, *attR* and *attB*, were cleaved with a restriction endonuclease and resolved by electrophoresis in

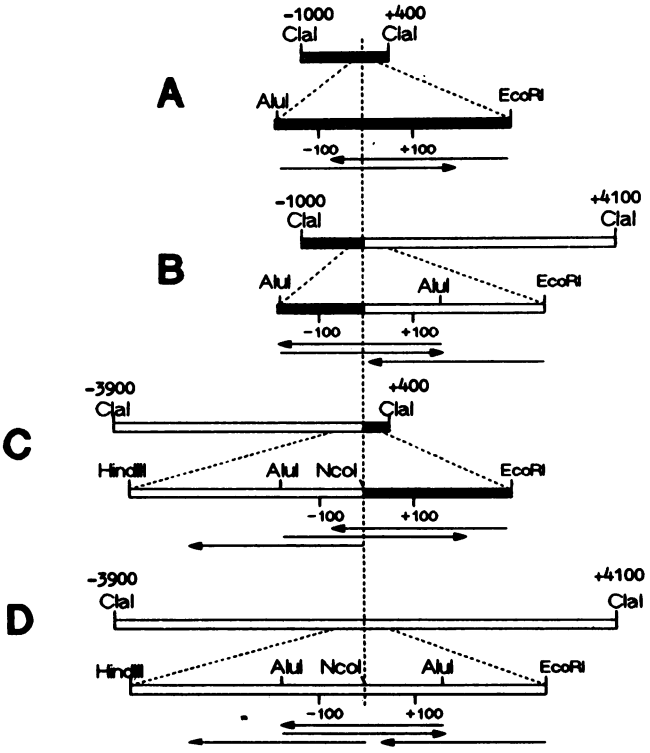

FIG. 1. Cloned restriction fragments of the genomes of *S. aureus* 8325-4 and its φ11-lysogenized strain and of the genome of bacteriophage φ11 containing the attachment sites. The DNA sequencing strategy is indicated. (A) *attB*; (B) *attL*; (C) *attR*; (D) *attP*. Expanded restriction maps of the region containing each *att* site are also shown. Arrows indicate the direction of sequencing. The vertical broken line indicates the approximate location of the center of the core. Numbers represent the distance (in base pairs) from the center of the core sequence.

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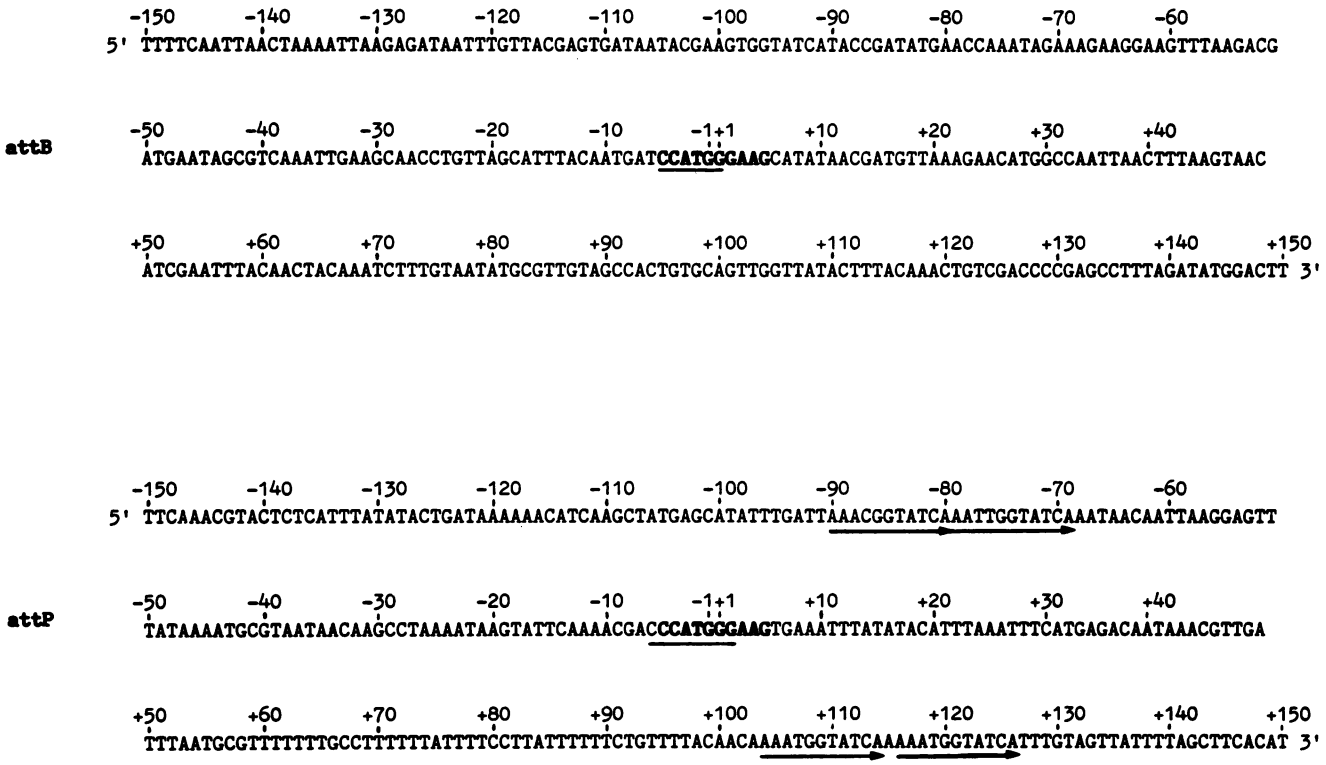

FIG. 2. Nucleotide sequences of the regions containing the *attB* and *attP* sites. Sequences are numbered from the center of the core; the base immediately to the right is +1, and the base immediately to the left is −1. The core sequences are shown in boldfaced type. Underlined sequences are the palindromic sequences discussed in the text. Arrows indicate the direct repeats.

adjacent gel lanes. The restriction profiles were compared, and the unique fragments were identified to contain the crossover region. These *att* site-containing fragments were isolated and cloned in the M13 bacteriophage derivatives mp18 and mp19 (16). The nucleotide sequences were then determined by the dideoxy chain termination method of Sanger et al. (13). The restriction maps and the sequencing strategy for the DNA segments carrying the *att* sites are shown in Fig. 1. The nucleotide sequences of the regions containing the *att* sites are shown in Fig. 2.

A 10-base-pair (bp) core sequence common to all four attachment sites was found. This feature was similar to the coliphage lambda *att* sites, in which the core is 15 bp (4), and to the staphylococcal phage L54a *att* sites, in which the core is 18 bp (5). It has been shown that the recombination of lambda and its host occurs within the core sequence (8). The existence of the core sequence implies that the crossover of the φ11 DNA and its host chromosome is within the 10 bp. The core regions of phages φ11 and L54a share a 6-bp homologous sequence (Table 1). Since both core sequences are very short, the homology is significant. It indicates that the site-specific recombination system of both phages may be similar.

A high A+T content has been proposed to promote DNA recombination (15). In lambda, the *attP* site has a high A+T content (4). However, in φ11 and L54a, the A+T content of the *attP* region is similar to that of an average staphylococcal chromosome and of an average phage genome (5, 10, 11).

DNA sequence features such as palindromic sequences, inverted repeats, and direct repeats are the potential protein-binding sites for proteins involved in recombination. A computer search indicated that *attP* contains more of these features than does *attB* (data not shown), suggesting the *attP* may play a more important role. This possibility is in accordance with lambda site-specific recombination, in which *attP* provides more protein-binding sites than does *attB* (15). One of these features of φ11 that is worth mention is the palindromic sequence, CCCATGGG in *attP* and CCATGG in *attB*, found in the core region (Fig. 2). This palindrome is located at the core and might serve as the cleavage site for recombinase in a manner similar to that of a restriction endonuclease cleavage site. The other feature that is also worth noting is an 11-bp nearly perfect direct repeat found in *attP* at positions −75, −85, +110, and +120 (Fig. 2). This direct repeat may represent the binding site for recombinase because (i) this arrangement is similar to that of

TABLE 1. Comparison of *attP* of bacteriophages φ11 and L54a

Phage	Core[a]	Direct repeat[b]	Positioning of direct repeat
φ11	CCATGGGAAG	AAQYGGTATCA	−75, −85, +110, and +120
L54a	ATCATACAAGGATGGGAT	AAAAAGGGCARA	−130, −110, +80, and +90

[a] Underlining indicates homology.
[b] Q = A or T; Y = C or T; R = A or G.

the integrase-binding site in lambda *attP* and (ii) this arrangement is also similar to that of a 12-bp direct repeat found in the L54a *attP* site (Table 1 and reference 5). Deletion of the direct repeat of L54a *attP* abolished its ability to serve as a functional recombination site, implying that it is required for recombination and may serve as the protein-binding site (unpublished data).

In summary, we have reported the sequences of the attachment sites involved in phage φ11 integration and excision in *S. aureus*. The system is very similar to that of phage L54a. The similarity of the attachment sites together with the common properties of these two phages (i.e., genome size, organization, and immunity) suggests that these two phages evolved from a common ancestor.

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