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Location of the *Bacillus subtilis* Temperate Bacteriophage ϕ105 *attP* Attachment Site

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Chromosomal DNAs of lysogens of ϕ105 and ϕ105 DI:1t were digested with restriction enzymes *Eco*RI and *Hpa*I and were probed with nick-translated mature ϕ105 DNA. Altered bacteriophage-specific bands in the lysogens were detected, indicating that the phage integrates into the host chromosome at a single site, probably via a Campbell-type circular intermediate. The phage attachment site is centrally located in the phage genome and lies between the phage immunity region and the nonessential deletable region of ϕ105.

Temperate *Bacillus subtilis* bacteriophage ϕ105 integrates into the host chromosome at a unique site located between the *pheA* and *ilvC* bacterial markers (3, 8). It has been suggested that the phage attachment site lies at the ends of the mature phage (1, 2, 3, 9) in contrast to the internal *att* site of the prototypic *Escherichia coli* temperate phage lambda.

The lack of any confirmed ϕ105 *att* mutations prevents the precise genetic localization of the ϕ105 *att* site. We attempted to physically locate the ϕ105 *att* site by analyzing the phage-specific DNA sequences contained in ϕ105 lysogens. Lysogen bands showing mobilities different from their mature phage counterparts were detected, allowing localization of the ϕ105 *att* site.

Lysogens of ϕ105 and the turbid plaque-deletion mutant DI-1t (5) were made by infection of strain YB886: *trpC2*, *metB5*, *xin*-1, $SP\text{-}\beta$ (11) as previously described (4). The ϕ105 lysogen, available from the *Bacillus* Genetic Stock Center, The Ohio State University, Columbus, Ohio, as BGSC 1L32, and the DI-1t lysogen (BGSC 1L34) were induced with mitomycin C (4) and purified on CsCl gradients (4), and the resulting phage was used as a source of mature phage DNA. Chromosomal DNA from BGSC 1L32, BGSC 1L34, and YB886 (BGSC 1A304) was isolated after sodium dodecyl sulfate lysis of early log (optical density at 660 nm of 0.15) lysozyme-treated cultures. The DNAs were restricted with *Eco*RI or *Hpa*I (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) (Fig. 1) according to the recommendations of the manufacturer. Fragments were separated on 0.4% agarose gels and were transferred to Zeta Probe membranes (Bio-Rad Laboratories, Richmond, Calif.) in a Bio-Rad transblot cell. Transfer was for 16 h at 40 V in TAE (Tris-acetate, 0.04 M; EDTA, 0.002 M) buffer as described by the manufacturer (Bio-Rad Laboratories, Richmond, Calif.). Nick-translated ϕ105 DNA was prepared by using the Bethesda Research Laboratories nick-translation kit and [^{32}P]CTP (Amersham Corp., Arlington Heights, Ill.). Hybridization was performed under stringent conditions as described in the *Bio-Rad Transblot Cell Manual*, Bio-Rad Laboratories.

The results of these hybridizations are shown in Fig. 1A and B. Shifted bands are observed because of the integration of the wild-type and deleted phage into the chromosome at centrally located restriction fragments. The molecular weights of the phage-specific bands were determined to accurately locate the shift of phage fragments joined to chromosomal DNA by integration (data not shown). The *Eco*RI restriction pattern of lysogens 1L32 and 1L34 indicates that the phage *att* site cannot be located near the ends of the phage. It is clear that the end-joint fragment *Eco*RI-A is present in both lysogens. The end fragments that make up

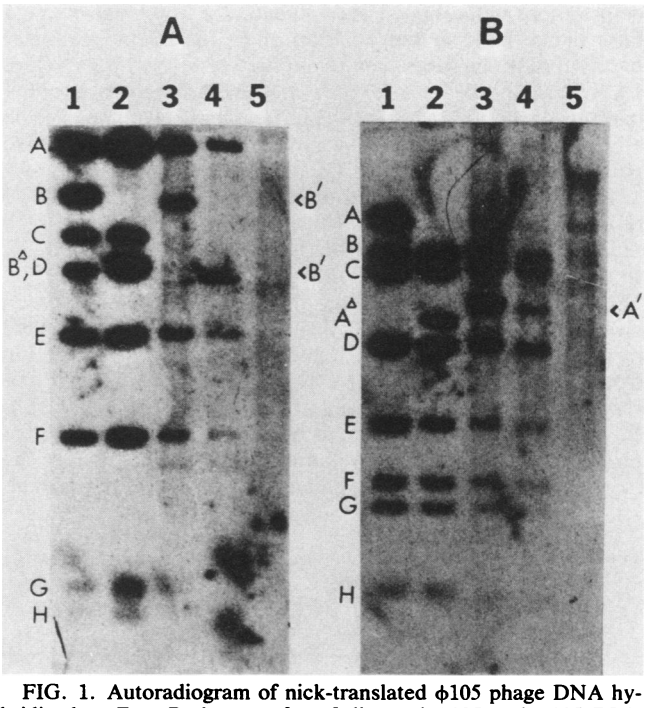

FIG. 1. Autoradiogram of nick-translated ϕ105 phage DNA hybridized to Zeta Probe transfer of digested ϕ105 and ϕ105 DI:1t lysogen DNAs. Lanes: 1, ϕ105 DNA; 2, ϕ105 deletion, DI:1t; 3, lysogen of ϕ105, BGSC 1L32; 4, lysogen of ϕ105 deletion DI-1t; 5, YB886 DNA (non-lysogen). Shifted bands caused by deletions in *Eco*RI-B and *Hpa*I-A are indicated by B^{Δ} and A^{Δ}, respectively. Shifted bands caused by the integration of phage DNA into chromosomal DNA are indicated by B′ and A′ to the right of panels, respectively.

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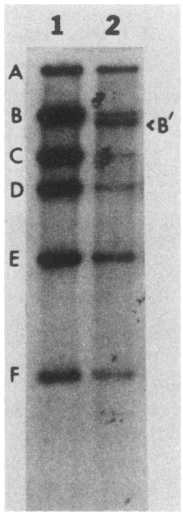

FIG. 2. Hybridization of nick-translated ϕ105 to ϕ105 mature phage DNA (lane 1) and to strain BGSC 1L32 chromosomal DNA (lane 2) isolated from stationary-phase cells. Both DNAs are digested with *Eco*RI.

the end joint (*Eco*RI-C and *Eco*RI-D) are absent in both lysogens. The ϕ105 *Eco*RI-B bands show a slight reduction in size in the corresponding lysogen 1L32, from approximately 11.0 kilobases (kb) (Fig. 1A, band B, lane 1) to approximately 10.7 kb (Fig. 1A, band B′, lane 3). This shift is more clearly demonstrated in Fig. 2, which shops hybridization to ϕ105 lysogen DNA isolated from stationary-phase cells. These cells, which shed phage at a low level, show a faint phage band at the position of *Eco*RI-B and a shorter band running slightly faster. A similar shift, from 7.8 kb (Fig. 1A, band B′, lane 3) to 7.35 kb, is seen in the corresponding band from the DI:1t lysogen (Fig. 1A, band B′, lane 4). The attachment site of ϕ105 apparently lies near (within 400 base pairs) one end of the *Eco*RI-B fragment.

This result was confirmed by examination of *Hpa*I digests of lysogens 1L32 and 1L34 (Fig. 1B). The largest wild-type *Hpa*I band, *Hpa*I-A, spans the right side of *Eco*RI-B (Fig. 3). In the wild-type lysogen, *Hpa*I-A is split by integration (Fig. 1B, band A′, lane 3) into two bands of approximate equal sizes (6.16 and 6.0 kb; Fig. 1B, band A′, lanes 3 and 4). One of these two bands (6.16 kb) is missing because of deletion from 1L34 (Fig. 1B, lane 4), as is expected from the restriction map. In both cases, the end-joint fragment (*Hpa*-B, 8.0 kb) is present. Thus, it appears that the *att* site for phage ϕ105 is located near the end of the right side of the *Eco*RI-B band. The 2-kb fragment, reported by Guillen et al. (6) to be characteristic of ϕ105 integrated into the bacterial chromosome, may represent the rightmost junction fragment of prophage and chromosome.

The region containing the right end of *Eco*RI-B, where we suspect ϕ105 *attP* to be located, has recently been cloned and sequenced (2a). Comparison of this sequence with the lambda *attP* core (10) reveals extensive homology (13 of 15 bases). There is no reason at the present time to suspect that the ϕ105 *att* site should be similar to that of lambda.

The positioning of ϕ105 *attP* (Fig. 3) at the right side of *Eco*RI-B band, places it between the immunity region and the nonessential deletable region. This is remarkably similar to the location of *attP* in the lambda genome. Indeed the overall organization of ϕ105 now appears to be very similar to that of lambda.

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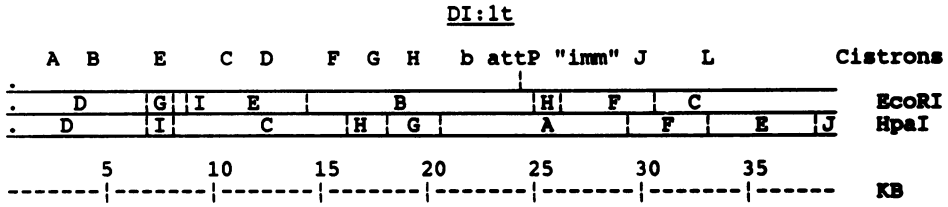

FIG. 3. *Eco*RI and *Hpa*I restriction enzyme map and genetic map of ϕ105 (7), showing the location of the ϕ105 attachment site (*attP*). Distance markers are shown (kb).