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Analysis of Recombination Occurring at SLP1 *att* Sites

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SLP1^{int} is a conjugative *Streptomyces coelicolor* genetic element that can transfer to *Streptomyces lividans* and integrate site specifically into the genome of the new bacterial host. Recombination of SLP1 previously has been shown to occur within nearly identical 112-base-pair *att* sequences on the plasmid and host chromosome. We report here that both integrative recombination and intermolecular transfer of SLP1^{int} require no more than a 48-base-pair segment of the *att* sequence and that SLP1 transfer occurs by a conservative rather than a replicative mechanism. The functions responsible for the excision of the element as a discrete DNA segment are induced during the conjugal transfer of SLP1.

The SLP1 genetic element, which exists in nature as an integrated sequence (i.e., SLP1^{int}) in the *Streptomyces coelicolor* chromosome, is capable of transfer into *Streptomyces lividans* and integration into a specific site on the *S. lividans* genome (1, 27). SLP1-mediated recombination events associated with transfer and integration occur at the attachment sites *attP* of SLP1 and *attB* of the host chromosome and at *attL* and *attR*, the recombination loci that flank the integrated element. The *att* sites are nearly identical 112-base-pair (bp) sequences; a 1-bp difference occurring at position 95 yields a *Bcl*I site in *attB* and *attR* that is absent from *attP* and *attL* (Fig. 1). We previously have proposed (27, 28) that SLP1^{int} is transferred between species as a transiently existing, physically autonomous 17-kilobase (kb) plasmid that integrates into the *attB* site of the recipient upon entering the cell. Autonomously replicating SLP1-derived extrachromosomal elements (1, 27, 28) can result from deletion of segments of the SLP1 sequence that are required either for integration or for maintenance of SLP1 in the integrated state.

Examination of the DNA sequences of the four SLP1 *att* sites reveals two regions of dyad symmetry (i.e., inverted repeats) (29) (Fig. 1). In several other systems, such inverted repeat sequences serve as loci for site-specific recombination (3, 9, 12, 13, 17, 18). Both inverted repeats in the SLP1 *att* segments lie in the region where SLP1-mediated recombination events are known to occur (29). We report here the results of insertion and deletion analyses that identify regions of the *att* sites essential for SLP1-mediated recombination and localize more precisely the actual site of recombination. We also provide direct evidence that intermolecular transfer of SLP1^{int} involves conservative excision of the 17-kb SLP1 sequence as a discrete DNA segment and show that the functions responsible for such excisive recombination are regulated rather than constitutively expressed.

MATERIALS AND METHODS

Bacterial strains and plasmids. The plasmids pACYC177 (5), pUC18, and pUC19 (37) were used as vectors for cloning in *Escherichia coli*, and the pIJ101-derived replicons pIJ364 (22) and pIJ702 (19) were used in *S. lividans*. *E. coli* MC1061 (4), *S. lividans* TK64 (16), and *S. lividans* 1326 (15) were used as cloning hosts. *S. lividans* C37, a derivative of 1326 that contains the SLP1-derived pCAO106 plasmid (30) inserted in its chromosomal *attB* site, was used for integrative recombination assays (see below).

For integrative recombination experiments, *Streptomyces* plasmids that contain SLP1 *att* sites were constructed (Fig. 2) by using an *attP* sequence obtained from pCAO112 (29) as a 115-bp *Bss*HII-*Hin*cII fragment or an *attB* sequence obtained from pCAO109 (29) as an 870-bp *Pst*I-*Sac*I fragment. pSCL250 and pSCL251 were used to test recombination capabilities of SLP1 sequences other than *attP* (Fig. 2). The SLP1 fragments included in these plasmids are a 542-bp *Bgl*II and a 314-bp *Bgl*II-*Pst*I fragment, respectively. These map between 10.6 and 11.5 kb on the pCAO106 circular map (30). Restriction sites at the ends of the inserts (Fig. 2) were derived from the polylinker in the pUC18-derived plasmids used as intermediates in the cloning procedure. pCAO1000 contains the *attP* fragment inserted into the pUC18 *Sma*I site, and pCAO1003 contains the *attB* fragment inserted into the pUC18 *Pst*I-*Sac*I sites.

Bacteriological methods. *E. coli* cells were grown at 37°C either on L agar or in L broth (24). *S. lividans* cells were grown at 30°C on R2YE agar plates or in YEME broth (14). *E. coli* cells were transformed as described previously (7), and transformants were selected on L media containing either 30 or 50 μg of ampicillin per ml. For *S. lividans* transformation, cells were grown in YEME containing 1% glycine. Protoplasts were made and transformed as described previously (20). Transformants were selected by overlaying the regenerating protoplasts, 12 to 16 h after plating, with P medium (20) supplemented with 0.7% agar and 500 μg of thiostrepton per ml. Thiostrepton was the kind gift of S. J. Lucania, E. R. Squibb & Sons, Princeton, N.J.

DNA manipulations. Total DNA was isolated from *S. lividans* by the method of Marmur (25). Large-scale preparations of *E. coli* or *S. lividans* plasmid DNA were made by the alkaline lysis method (24), and minipreps were made as described previously (2, 21).

Restriction enzymes purchased from New England BioLabs, Inc., Beverly, Mass., or from Bethesda Research Laboratories, Inc., Gaithersburg, Md., were used as specified by the manufacturers. Restriction fragments were transferred to BA-85 nitrocellulose membranes as described by Southern (34). The Bethesda Research Laboratories 1-kb ladder was used as a size marker. *E. coli* cells for colony

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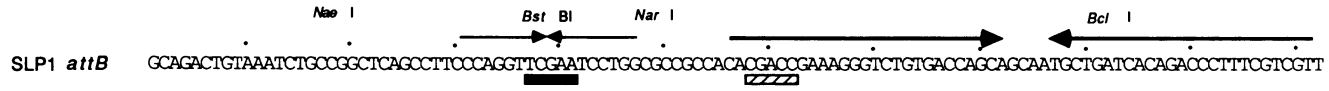

FIG. 1. DNA sequence of the SLP1 *attB* and *attR* sites (29). The *attP-attL* sequence is identical to the one shown, except for the absence of the *Bcl*I cleavage site due to a 1-bp substitution at bp 95 (A to G). Restriction endonuclease cleavage sites are indicated above the sequence. Inverted repeats of interest are indicated by bold or thin arrows. Two distinct segments of sequence conservation between the SLP1 *att* sites and those of other actinomycete site-specifically integrating elements are indicated by underlining (▬, ▨).

hybridization were grown on nitrocellulose membranes and lysed as described previously (10).

DNA ligation, filling in of the ends of DNA fragments with the Klenow fragment of *E. coli* DNA polymerase I, and addition of synthetic linkers were accomplished as described previously (24). DNA sequences were determined by the dideoxy-chain termination method (33).

Preparation of radioactively labeled DNA probes and hybridization to membrane-bound DNA. DNA probes were labeled with ^{32}P by nick translation (32). Filters were prehybridized at 55°C in a solution containing 50% formamide and 5× SSPE (1× SSPE is 1 mM disodium EDTA, 5 mM NaOH, 10 mM NaH_2PO_4, and 180 mM NaCl [pH 7.0]). The prehybridization buffer also contained Denhardt solution at a final concentration of 5× (8). Hybridization was performed under the same conditions as prehybridization, except that the hybridization buffer contained 10^6 cpm of radioactively labeled probe per ml. After hybridization, filters were washed three times (15 min per wash) in 5× SSPE–0.1% sodium dodecyl sulfate at 55°C. Hybridization was detected by autoradiography with XAR-5 film (Eastman Kodak Co., Rochester, N.Y.).

Assays for SLP1 integrative and excisive recombination. The assay for integrative recombination involved the use of pCAO106, a plasmid constructed by joining the entire SLP1 element to pACYC177 at the single *Bam*HI site of each molecule (29, 30). The integration and transfer properties of pCAO106 are similar to those of the native SLP1 element lacking the pACYC177 insertion (30; S. C. Lee, unpublished observations). Integration test plasmids (Fig. 2) were constructed by introducing modified or native *att* sequences or other SLP1-derived DNA fragments into the high-copy-number *Streptomyces* cloning vector pIJ364 or pIJ702. Figures 3 and 4 include partial mapping data for pCAO106; a detailed pCAO106 restriction map has been published previously (30).

The ability of various cloned DNA fragments to recombine with SLP1 during conjugal transfer was tested by mating a recipient *S. lividans* strain containing the test plasmid with the donor strain *S. lividans* C37 (30), which contains an integrated pCAO106 at the chromosomal *attB* site, and then determining whether a composite extrachromosomal replicon consisting of both the test plasmid and pCAO106 was formed. This was done by streaking an inoculating loop of C37 spores onto a lawn of *S. lividans* TK64 containing the test plasmid. After 72 to 84 h of growth on solid media, spores and mycelia were isolated from regions of confluent lethal zygosis (i.e., regions of SLP1 transfer [1]) and added to 50 ml of thiostrepton-supplemented (20 μg/ml) YEME broth. Plasmid DNA was isolated from these liquid cultures after 72 to 84 h of growth and used to transform a *recA* derivative of *E. coli* MC1061 (the gift of D. Van den Berg) to ampicillin resistance. Since the integration test plasmids contain no *E. coli* replicons (Fig. 2), sequences homologous to pIJ702 recovered from *E. coli* necessarily had become linked to pCAO106 by recombination. Such composite (i.e., recombinant) replicons were detected in *E. coli* transformants by colony hybridization to ^{32}P-labeled pIJ702 DNA. Transformed *E. coli* colonies were also tested for homology to SLP1 restriction fragments other than *attP*.

We tested the ability of pCAO106 to excise from the composite replicons formed during the integration assay. *S. lividans* TK64 protoplasts were transformed with composite replicons, and Ts^r transformants were selected. After 4 to 5 days of growth of these transformants, spores and aerial mycelia were pooled and used to inoculate 50 ml of YEME supplemented with 20 μg of thiostrepton per ml. Plasmid and chromosomal DNA were isolated from the broth cultures after they had grown for 3 to 4 days. The DNAs were then subjected to restriction endonuclease digestion and Southern blot hybridization analysis to determine whether the pCAO106 segment of the composite replicons had excised.

RESULTS

Less than 50 bp of the *att* sequence functions as *attB*. Table 1 presents the results of integrative recombination experiments using the test plasmids shown in Fig. 2. Test plasmids containing unmodified *attB* or *attP* sites (pSCL229 and pCAO301, respectively) recombine with pCAO106 with similar efficiencies, despite the 1-bp difference in the *att* site sequence. Additionally, test plasmids containing *att* sites recombined with pCAO106 much more efficiently than did plasmids that included considerably larger SLP1 DNA fragments lacking an *att* site (pSCL250 and pSCL251 [Fig. 2; Table 1]).

Insertions into either the *Bcl*I(pSCL203) or *Nar*I (pCAO303) sites had modest, if any, effects on the frequency of integrative recombination. However, insertions into either the *Bst*BI(pSUM27) or *Nae*I(pSCL277) sites reduced and abolished, respectively, the ability of the test plasmid to recombine with pCAO106. Thus, the segments of the *att* site that are important in recombination are located near the left end of the *att* sequence as drawn in Fig. 1. Furthermore, sequences to the left of the *Nar*I site not only are necessary for the cloned *att* sites to function, but also are sufficient; deletions to the right of the *Nar*I site did not prevent integrative recombination (Table 1, pSCL233 and pSCL234).

The effect of insertions in the *Bst*BI site on integrative recombination (pSUM27) suggested that the region of dyad symmetry centered about this restriction site (Fig. 1) is involved in integrative recombination. However, the dyad centered on the *Bst*BI site was insufficient to participate in recombination, as shown by the inability of pCAO106 to integrate into pSCL278, a test plasmid containing a synthetic oligonucleotide comprising solely that inverted repeat (Fig. 2; Table 1).

Integrative recombination of SLP1 occurs to the left of the *Bst*BI site in the *att* sequence. The structures of the composite replicons present in 10 independent *E. coli* transformants were characterized by restriction and Southern blot analyses for each of the integratively competent test plasmids (Table 1). Restriction maps of the composite molecules, which were

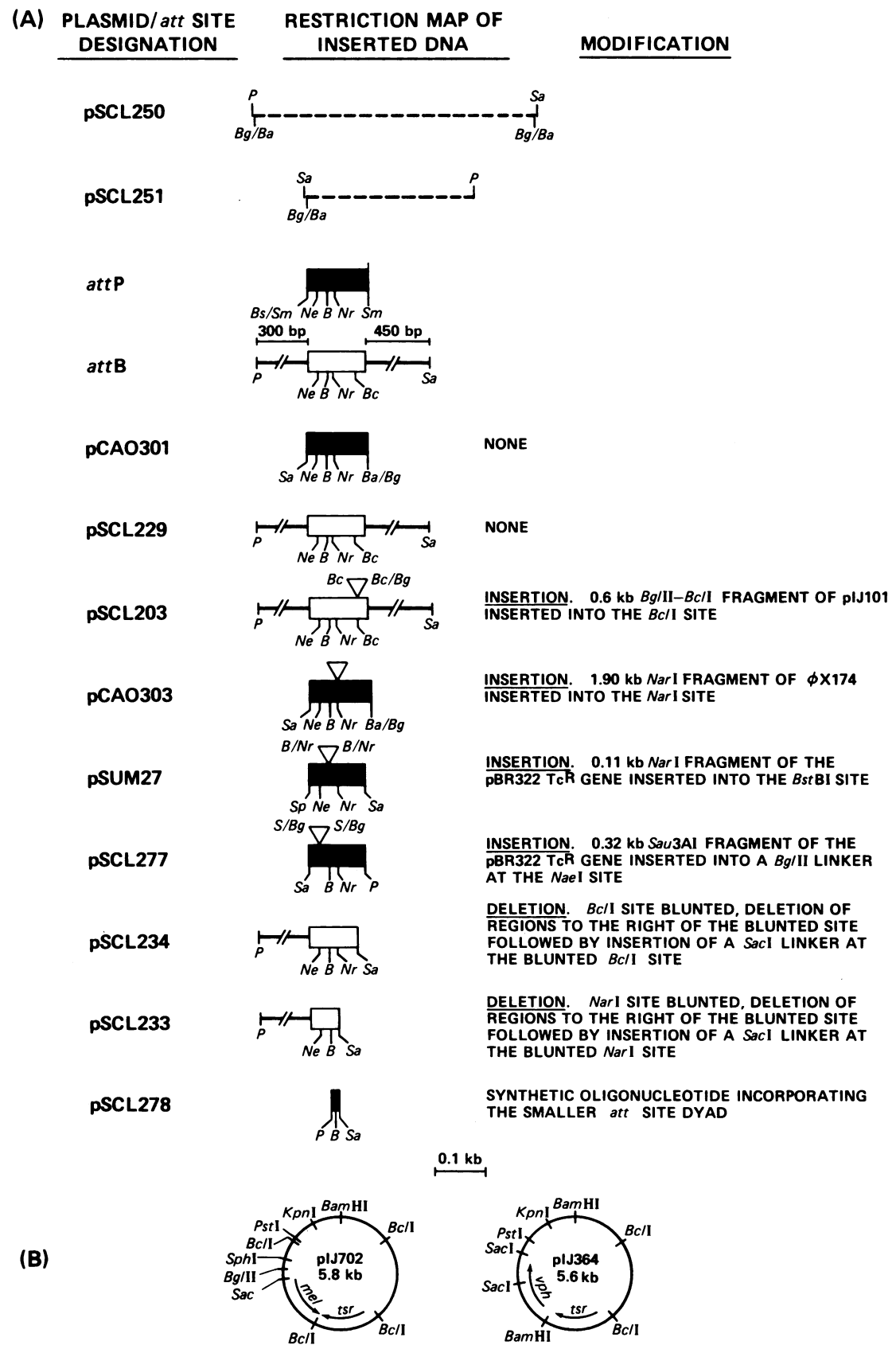

FIG. 2. (A) Restriction maps of cloned DNA fragments tested for their ability to undergo integrative recombination with pCAO106 during conjugal transfer. The fragments were introduced into *Streptomyces* cloning vectors by using the restriction sites shown at their termini. Symbols: ■, inserts derived from SLP1 *attP*; □, inserts derived from *attB*; ———, *S. lividans* chromosomal DNA flanking *attB*; - - - -, non-*attP* SLP1 DNA. Modifications of *att* site DNA are described at right. Restriction site abbreviations: *Ba*, *Bam*HI; *Bc*, *Bcl*I; *Bg*, *Bgl*II; *B*, *Bst*BI; *Ne*, *Nae*I; *Nr*, *Nar*I; *P*, *Pst*I; *Sa*, *Sac*I; *Sp*, *Sph*I. Lengths are indicated in kilobases. For all plasmids except pSCL234 and pSCL233, pIJ702 was used as the *Streptomyces* cloning vector. For pSCL234 and pSCL233, pIJ364 was used as the *Streptomyces* cloning vector. (B) Cloning vectors used for inserts shown in panel A. Note that in pSCL234, the juncture between the insert and vector occurs at the *Sac*I site within the pIJ364 *vph* gene. In pSCL233, the analogous juncture occurs at the *Sac*I site outside the *vph* gene. Genes indicated: *mel*, tyrosinase; *tsr*, thiostrepton resistance; *vph*, viomycin phosphotransferase. Adapted from reference 14.

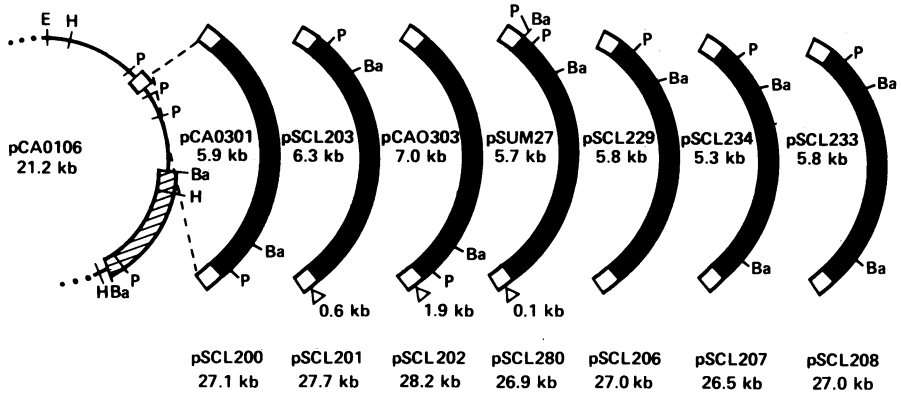

FIG. 3. Restriction maps of composite replicons. An arc of the pCAO106 map is shown at left to indicate the orientation of integration of pCAO106 into each composite replicon. Integration test plasmid DNAs are shown as inserts into the pCAO106 *attP* site. Symbols: ▭, *attP* or *attB* DNA; ———, non-*attP* SLP1 DNA; ▨, pACYC177 replicon; ▬, test plasmid cloning vector sequences; △, insertion mutations of integration test plasmids. The names of integration test plasmids are shown within the arcs of their maps, while the names of the corresponding composite replicons are shown below the maps. Sizes are indicated in kilobases. Restriction endonuclease cleavage site abbreviations are as in Fig. 2A; E, *Eco*RI; H, *Hin*dIII.

uniform for any given test plasmid (data not shown), are presented in Fig. 3. Each of the composite replicons contained two recombinant *att* sites flanking the pCAO106 portion of the molecules. By determining which of these *att* sites included the insertion mutation present in the *att* site of the corresponding integration test plasmid (Fig. 2), we determined the position of recombination within the *att* sequence. The results of these experiments showed that insertions into the *Bcl*I, *Nar*I, or *Bst*BI sites of the *att* sequence (Fig. 2) ended up in the *attR* sites of the composite replicons (Fig. 3), implying that integrative recombination occurred to the left of the *Bst*BI site as the *att* sequence is drawn in Fig. 1.

The foregoing analysis is illustrated schematically (Fig. 4) for recombinations involving pSUM27, a test plasmid having an insert in the *Bst*BI site of its *att* sequence. Southern blot analyses (Fig. 5A) showed that recombination to the left of the *att* sequence *Bst*BI site resulted in the presence in composite replicon pSCL280 of restriction fragments of lengths that are the sum of the corresponding fragment lengths in pCAO106 and pSUM27 (Fig. 4).

Plasmids having homology to SLP1, but lacking homology to pIJ702, were obtained in every integration experiment (Table 1). At least one such plasmid was characterized for each integration test plasmid used in the experiments shown in Table 1. These plasmids were uniform in structure and were indistinguishable from pCAO106 (data not shown). During these experiments, we also observed that pSCL251, which contains a 300-bp SLP1 DNA fragment that lacks *attP*, yielded a single *E. coli* transformant containing DNA homologous to pIJ702 (Table 1). We assume that this event resulted from homologous recombination with pCAO106. However, the restriction endonuclease cleavage pattern of the plasmid in that transformant was not as expected if the plasmid had resulted from a single recombination event between pCAO106 and pSCL251 (data not shown). Either the plasmid was formed by multiple recombination of events or, alternatively, it had undergone a rearrangement after its formation.

pCAO106 can excise from composite replicons, and excision occurs to the left of the *att* sequence *Bst*BI site. The composite replicons in Fig. 3 were subjected to the excision assay described in Materials and Methods. When the composite replicons were introduced into *S. lividans* TK64 by transformation, plasmids identical to the corresponding integration test plasmids were produced, as determined by restriction digestion (*Bcl*I, *Pst*I, and simultaneous digestion with *Pst*I and *Sac*I [data not shown]). These data indicate that the *att* site modifications that permitted integrative recombination (Table 1; Fig. 2) also allowed excision of the entire pCAO106 DNA segment from the composite replicons. Furthermore, these data show that excisive recombination, like integrative recombination, occurred to the left of the *Bst*BI site of the *att* sequence. Figure 5B substantiates this point for the excision of pCAO106 from composite replicon pSCL280. Comparison of the restriction patterns and Southern blots of pSUM27 to the plasmid resulting from the excision of pCAO106 from pSCL280 shows that the restriction endonuclease cleavage patterns of the two plasmids are indistinguishable.

The data in Fig. 5B show that excisive recombinations occur to the left of the *att* sequence *Bst*BI sites as drawn in Fig. 1. Southern blot hybridization with the *attP* sequence of pCAO1000 as a probe was used to test for recombinations occurring to the right of the *Bst*BI site (Fig. 5C). Such recombination events occurring in the excision of pCAO106 from pSCL280 would have produced a hybridizing fragment identical in size to the 149-bp *Pst*I-*Sac*I fragment of pCAO1000 that includes homology to the *attP* sequence. No such fragments were detected, whereas hybridizing fragments at a concentration of 0.1% that of the 259-bp band in Fig. 5C, lane 5 were easily detected in Southern blot exposures similar to that of Fig. 5 (Lee, unpublished).

It previously has been reported that integration of pCAO106 replicons into the chromosomal *attB* site of *S. lividans* occurs upon its introduction by transformation into the bacterial host. In the present experiments also, pCAO106 was not detected as a discrete replicon in the plasmid DNA fractions of *S. lividans* transformed with composite replicons (Fig. 5); however, Southern blot hybridization showed its presence in the chromosomal *attB* sites of those cells (data not shown). As expected, transformants receiving composite molecules produced lethal zygosis in matings to SLP1-minus *S. lividans*.

Excision of SLP1^{int} is suppressed during vegetative growth and is induced by mating. SLP1 is maintained extremely stably as an integrated element: it has not been possible to isolate SLP1-minus *S. coelicolor*, and excised SLP1 mole-

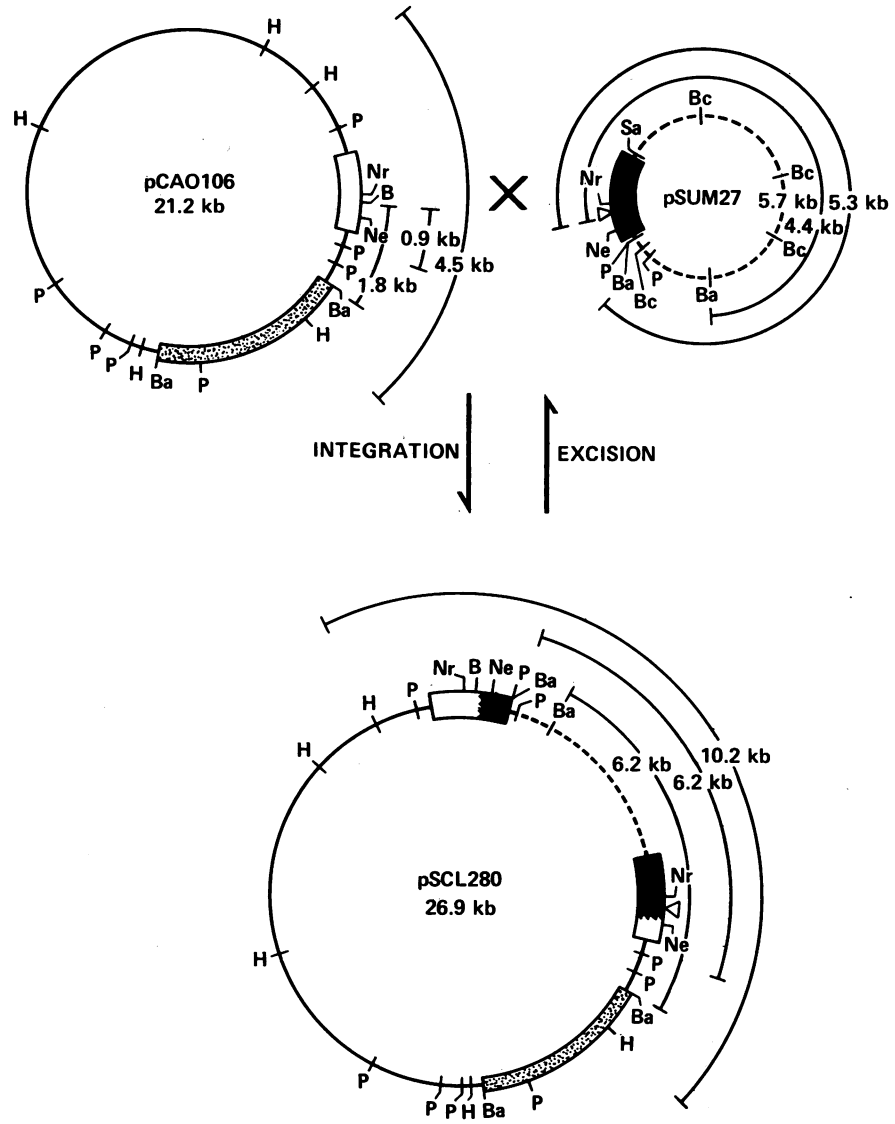

FIG. 4. Schematic depiction of integrative and excisive recombinations involving pSUM27. Sizes of restriction fragments used to determine the site- specificity of integrative recombination are indicated by arcs on the restriction maps. Only restriction sites used to analyze recombinations are shown. *Bcl*I and *Sac*I sites are shown on the pSUM27 map, since these sites were used to analyze the excision of pCAO106 from pSCL280 (see text and Fig. 5). *Nae*I, *Nar*I, and *Bst*BI sites of the plasmids are shown within *att* sequences (but not in any other portion of the replicons) to indicate the relative orientations of the attachment sites. Restriction site abbreviations are as in Fig. 2 and 3. Symbols: △, insertion mutations in pSUM27 and pSCL280 *att* sequence *Bst*BI sites; ———, the SLP1-derived DNA segment of pCAO106; - - - - -, pIJ702-derived sequences of pSUM27; ■, *attP* site of pSUM27; □, *attP* site of pCAO106; ▩, pACYC177-derived segment of pCAO106. Note that plasmids are not drawn to scale and that *att* sites are shown larger than scale for clarity.

cules were not observed in SLP1^{int} strains of *S. lividans*. However, free molecules of the SLP1 derivative plasmid pCAO106 were isolated from cultures that recently had undergone conjugal transfer of the integrated form of this element (Table 1). Any loss of SLP1^{int} resulting from excision during cell growth in the absence of conjugation might not have been observed because of rapid reintegration of excised SLP1 or because of reintroduction of the element by conjugal transfer. Alternatively, excision of SLP1^{int} might be suppressed in the absence of conjugal transfer. To investigate these possibilities, we used an experimental scheme analogous to that used for the assay of integrative recombination.

Strains used in these experiments contained the integration test plasmid pCAO301 or pSCL229 (Fig. 2) to act as a catcher for excised or conjugally reintroduced pCAO106. *S. lividans* C37 (*S. lividans* 1326 containing the chromosomally integrated pCAO106 plasmid) was mated to either *S. lividans* C39 (1326 containing pSCL229 [Fig. 2]) or *S. lividans* C40 (1326 containing pCAO301 [Fig. 2]), and spores taken from regions of lethal zygosis were used as inocula for the isolation of plasmid DNA for transformation of *E. coli* MC1061 to Ap^r (Table 2). Plasmid DNA was also isolated from similar amounts (about 20 mg [wet weight]) of unmated cultures of *S. lividans* C37 and C38 (C37 containing pSCL229). DNA isolated from recently mated *S. lividans* yielded at least a 10^3-fold-higher frequency of *E. coli* transformants than were observed for unmated cells (Table 2).

TABLE 1. Ability of cloned DNA to function as *attB* sites during conjugal transfer of pCAO106[a]

Test plasmid	pIJ702/SLP1 ratio[b]		% Composite replicons
	Expt 1	Expt 2	
pSCL250	0/100	ND[c]	0
pSCL251	1/100	ND	1
pCAO301	127/172	332/400	80
pSCL229	22/35	18/21	71
pSCL203	247/306	157/216	79
pCAO303	55/80	20/41	62
pSUM27	10/100	2/22	10
pSCL277	0/100	0/27	0
pSCL234	23/45	55/100	54
pSCL233	41/99	ND	41
pSCL278	0/83	0/43	0
pIJ702	0/64	ND	0

[a] *S. lividans* strains containing the integration test plasmids shown at left were recipients in matings with *S. lividans* C37, which contains a chromosomally integrated pCAO106 plasmid (see Materials and Methods). Plasmid DNA was isolated from recently mated cells and used to transform a *recA* derivative of *E. coli* MC1061 to ampicillin resistance. Integrative recombination was detected by colony hybridization to the *E. coli* transformants by using pIJ702 as probe. Colonies were also hybridized to SLP1 DNA.

[b] The pIJ702/SLP1 ratio indicates the ratio of *E. coli* transformants that contain DNA hybridizing with pIJ702 probe to *E. coli* containing DNA hybridizing to SLP1 probe. Data presented for experiments 1 and 2 are from independent *S. lividans* matings.

[c] ND, Not done.

DISCUSSION

The experiments reported here indicate that the 1-bp sequence difference between the *attB-attR* and the *attP-attL* sequences (29) does not affect recombination between *att* sites; the cloned sites are functionally interchangeable. The segment of the *att* region essential for its optimal function straddles and extends to the left of the *Bst*BI site: integrative and excisive recombination occur within this segment, and insertions into this region of *att* severely impair recombination. Since all *att* sequences to the right of the *Nar*I site can be deleted without abolishing recombinational activity, the larger inverted repeat in the *att* region (Fig. 1, bp 57 to 111) cannot be the site of SLP1 recombination, as had been speculated previously (29). Rather, SLP1-mediated recombination may involve the *att* region dyad symmetry between bp 31 and 47 (Fig. 1). DNA insertion within this region of dyad symmetry impairs SLP1 recombination (Table 1, pSUM27), but this inverted repeat sequence alone is not sufficient for *att* site function (Table 1, pSCL277).

The SLP1 *att* sequence exhibits certain structural similarities to the attachment sites of other integrating genetic elements of actinomycetes (6, 15, 23, 26, 31). Inverted repeat sequences homologous to the dyad symmetry centered about the *Bst*BI site of the SLP1 *att* sequences are present in the *att* sites of pMEA100 (23, 26), pSAM2 (31; F. Boccard, personal communication), and pIJ408 (15; R. Hütter, personal communication). The sequence TCGAA is highly conserved between the homologous inverted repeats of the *att* site of SLP1 (Fig. 1) and those of the other elements. In addition, all four of the *att* sites contain a second conserved pentameric sequence, CGACC, which lies outside the conserved inverted repeats (Fig. 1). Our experiments indicate that the conserved CGACC is dispensable for SLP1-mediated recombination, as is more than half of the total length of the 112-bp *att* sequence. Similarly, in the yeast 2μm circle, two perfect 599-bp inverted repeats are observed (11); however, the minimal substrate for *flp*-mediated recombination is only a 34-bp region of dyad symmetry (17) within the highly conserved arms of the inverted repeats.

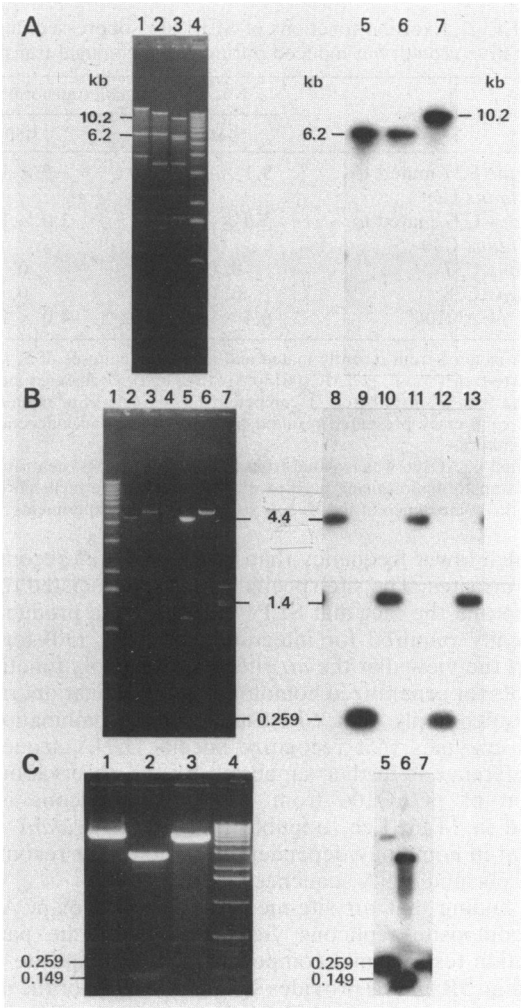

FIG. 5. Restriction and Southern blot analysis of integrative and excisive recombinations involving pSUM27. The plasmid resulting from the excision of pCAO106 from pSCL280 is referred to as pSCL280 excision plasmid. Fragment sizes are indicated. (A) Lanes: 1, pSCL280, *Bam*HI digest; 2, pSCL280, *Pst*I digest; 3, pSCL280, *Hin*dIII digest; 4, Bethesda Research Laboratories 1-kb sizing ladder; 5 to 7, Southern blot of plasmid digests as in lanes 1 to 3 probed with the 0.11-kb *Nar*I fragment that is inserted into the pSUM27 *att* sequence *Bst*BI site (Fig. 2). (B) Lanes: 1, Bethesda Research Laboratories 1-kb sizing ladder; 2, pSCL280 excision plasmid, *Bam*HI digest; 3, pSCL280 excision plasmid, *Pst*I-*Sac*I digest; 4, pSCL280 excision plasmid, *Bcl*I digest; 5, pSUM27, *Bam*HI digest; 6, pSUM27, *Pst*I-*Sac*I digest; 7, pSUM27, *Bcl*I digest; 8 to 13, Southern blot of plasmid digests as in lanes 2 to 7, probed with the 0.11-kb *Nar*I fragment that is inserted into the pSUM27 *att* sequence *Bst*BI site (Fig. 2). (C) Lanes: 1, pSCL280 excision plasmid, *Pst*I-*Sac*I digest; 2, pCAO1000, *Pst*I-*Sac*I digest; 3, pSUM27, *Pst*I-*Sac*I digest; 4, Bethesda Research Laboratories 1-kb sizing ladder; 5 to 7, Southern blot of plasmid digests as in lanes 1 to 3 probed with a 149-bp fragment of a pUC18 derivative (i.e., pCAO1000) that contains the *attP* sequence (see text). The high-molecular-weight hybridizing signal in panel C, lane 6, is due to vector contamination of the probe. The large pIJ702 fragment of pSUM27 and pSCL280 show minor cross-hybridization with the probe (panel C, lanes 5 and 7).

Recombination between other homologous sequences of SLP1 (compare pSCL250 and pSCL251 in Table 1) occurred

TABLE 2. Excision functions of SLP1 are suppressed during vegetative growth but induced during SLP1 conjugal transfer[a]

Source of DNA	No. of Ap^r *E. coli* transformants	
	Expt 1	Expt 2
S. lividans C37 mated to *S. lividans* C39	9.1×10^2	1.5×10^3
S. lividans C37 mated to *S. lividans* C40	5.0×10^3	2.0×10^4
S. lividans C37	0, 0, 0	0, 2
S. lividans C38	0, 0, 0	0, 0
Purified pCAO106[b]	8.4×10^5/μg	4.0×10^5/μg

[a] DNA isolated from recently mated and nonmated cultures of *S. lividans* was used to transform *E. coli* MC1061 to Ap^r (See text). Each experiment was performed with a single batch of competent *E. coli* cells. For nonmated *S. lividans*, each entry presented resulted from DNA from an independent *S. lividans* culture.

[b] Purified pCAO106 was isolated from *E. coli* by isopycnic centrifugation. Data on transformation using purified pCAO106 DNA are included here to indicate the competence of the *E. coli* MC1061 in these experiments.

at a much lower frequency than SLP1-mediated recombination at *att* sites. The site specificity of *att*-associated recombination and the fact that SLP1-encoded gene products are specifically required for integration of SLP1 (30) together support the view that the *att* sites do not simply function as hot spots for generalized homologous recombination; rather, our results imply that SLP1-mediated recombination involves enzymes that recognize specific DNA sequences. This inference is further supported by the observation that excision of pCAO106 from composite replicons is not blocked in JT46 (Lee, unpublished), an *S. lividans* strain deficient in homology-dependent intramolecular recombination of plasmid DNA sequences (35).

Our finding that *att* site-mediated excision of pCAO106 from composite replicons yielded precisely the parental integration test plasmid component of the composite molecules (Fig. 5B and C) provides strong evidence for the notion that excision of integrated SLP1 elements is conservative. In addition, isolation of free pCAO106 plasmid replicons that had excised from their initial chromosomal location during assays for integrative recombination (Table 2) supports the previously presented model (28) depicting intermolecular transfer of SLP1 as involving a circular full-length SLP1 molecule.

The observation that excision of SLP1^{int} is stimulated by conjugal transfer or by introduction of SLP1^{int}-containing composite plasmids into SLP1-minus cells by transformation is reminiscent of the process of zygotic induction observed when an F plasmid carrying a λ prophage is transferred to an F-minus recipient (36). Our results imply the existence of an SLP1-encoded regulatory moiety that ordinarily maintains SLP1 in the integrated state. The relationship of this putative regulator of SLP1 excision to the recently discovered *imp* function (S. R. Grant, S. C. Lee, K. J. Kendall, and S. N. Cohen, manuscript in preparation), which is encoded by a DNA segment deleted in naturally occurring SLP1-derived plasmids and which inhibits the ability of SLP1 to exist as an extrachromosomal replicon, remains to be determined.

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