Localization and Nucleotide Sequences of Genes Mediating Site-Specific Recombination of the SLP1 Element in *Streptomyces lividans*

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SLP1 is a 17.2-kbp genetic element indigenous to the *Streptomyces coelicolor* chromosome. During conjugation, SLP1 can undergo excision and subsequent site-specific integration into the chromosomes of recipient cells. We report here the localization, nucleotide sequences, and initial characterization of the genes mediating these recombination events. A region of SLP1 adjacent to the previously identified site of integration, *attP*, was found to be sufficient to promote site-specific integration of an unrelated *Streptomyces* plasmid. Nucleotide sequence analysis of a 2.2-kb segment of this region reveals two open reading frames that are adjacent to and transcribed toward the *attP* site. One of these, the 1,365-bp *int* gene of SLP1, encodes a predicted 50.6-kDa basic protein having substantial amino acid sequence similarity to a family of site-specific recombinases that includes the *Escherichia coli* bacteriophage λ integrase. A linker insertion in the 5' end of the cloned *int* gene prevents integration, indicating that Int is essential for promoting integration. An open reading frame (*orf61*) lying immediately 5' to *int* encodes a predicted 7.1-kDa basic peptide showing limited sequence similarity to the excisionase (*xis*) genes of other site-specific recombination systems.

Streptomyces are gram-positive filamentous soil bacteria notable for their complex morphology as well as the production of a variety of antibiotics and other secondary metabolites, many of which have medical or agricultural importance. Numerous extrachromosomal elements have been identified for these organisms, including a novel family of chromosomally integrated self-transmissible plasmidogenic genetic elements (reviewed in reference 35). SLP1, indigenous to the chromosome of *Streptomyces coelicolor* (6), was the first of this group to be identified. A model in which the integrated SLP1 element (i.e., SLP1^{int}) can excise from the *S. coelicolor* chromosome during conjugation with a strain lacking SLP1, transfer to the recipient strain as a transiently existing circular molecule, and either integrate into the recipient chromosome at a site identical to that occupied in the donor strain or undergo deletion of sequences that suppress autonomous replication and consequently be maintained as an autonomously replicating plasmid (33) has been proposed.

The recombination reactions mediated by SLP1 are site-specific and involve 112-bp regions of homology on SLP1 (*attP*) and the host chromosome (*attB*) that have only a single-base-pair difference (34). The integration event generates sites at the left (*attL*) and right (*attR*) of the integrated element that retain the homologies between *attP* and *attB*. Characterization of these sequences (29) has shown that the 5'-terminal 48-bp segment of *attB*, when cloned onto a high-copy-number plasmid in *Streptomyces lividans*, functions as *attB* in recombination with an incoming SLP1 element. This 48-bp segment of *attB* has been shown to contain the 3' portion of a tRNA^{Tyr} gene that is essential for viability of *S. lividans* (48).

Two SLP1 DNA segments in addition to *attP* have been implicated in the formation and/or maintenance of stable

integrants. A derivative of SLP1 containing an insertion at a *KpnI* site 1.3 kb from *attP* (Fig. 1), within a locus designated *intB*, fails to integrate. In addition, integration of SLP1-derived plasmids deleted for a second locus (i.e., *intA*, Fig. 1) situated at some distance from *attP* and *intB* was not observed. This defect could be complemented by providing the *intA* locus in *trans* on a plasmid unrelated to SLP1 (36).

We report here studies that further characterize the functions required for *att*-mediated integration and excision of SLP1. We find that a 2.2-kb region of SLP1 promotes integration. This region includes, in addition to *attP*, two potential open reading frames (ORFs), the predicted products of which show similarity to the excisionase and integrase of other site-specific recombination systems. Functional analysis of the *int* ORF indicates that its product is the only plasmid-encoded protein required for integration.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study were *Escherichia coli* BRL2288, a *recA56* derivative of MC1061 [F⁻ *araD139* Δ (*ara-leu*)7679 Δ (*lac*)X74 *galU galK hsdR2* ($r_K^- m_K^-$) *mcrB1 rpsL* (Str^r)] obtained from Life Technologies, Inc. (Gaithersburg, Md.), and for the isolation of undermethylated DNA, RM1602, a nonmucoid derivative of GM33 (*dam-3* F⁻) obtained from R. J. Meyer, and *S. lividans* 66 strains C14 (wild type) (33) and TK64 (*pro-2 str-6*) (26).

Bacteriological methods. *E. coli* was grown at 37°C on L agar or in L broth (40) supplemented as necessary with ampicillin (60 μ g/ml), kanamycin (25 μ g/ml), or G418 (50 μ g/ml). *S. lividans* was grown at 32°C on either R2YE agar (25) or on MY agar plates (10 g of malt extract, 4 g of yeast extract, 20 g of Bacto Agar per liter) supplemented with CuSO₄ to a final concentration of 2 μ M (27a) and, as necessary, with thiostrepton (50 μ g/ml), G418 (100 μ g/ml), hygromycin (200 μ g/ml), or viomycin (30 μ g/ml). Liquid cultures of *S. lividans* were grown in YEME broth (25) with

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FIG. 1. Map of SLP1. The circular form of SLP1 and the location of relevant restriction endonuclease cleavage sites are shown (36); bracketed numbers are used for reference in the text. DNA present in the naturally occurring deletion derivative SLP1.2 (6) is indicated by the solid black arc. Regions of SLP1 essential for replication and maintenance (speckled), or transfer (striped) (25, 46); the locus inhibiting plasmid maintenance, *imp* (21); and regions previously implicated in integration, *intA* and *intB* (open) (36), and the site of integration, *attP* (34), are indicated.

antibiotics at 10% of the above concentrations. Thiostrepton and viomycin were gifts from S. J. Lucania, E. R. Squibb & Sons, Princeton, N.J., and The Upjohn Co., Kalamazoo, Mich., respectively. Transformations of *E. coli* (12) and *S. lividans* (25) were as previously described. Protoplasts of *S. lividans* were overlaid after 12 to 18 h at 32°C with 1 ml of water containing antibiotics sufficient to yield the final concentrations described above, assuming a 25-ml volume.

DNA manipulations. Large-scale preparation of *E. coli* plasmid DNA was done essentially as described by Marko et al. (32) except that CsCl-ethidium bromide gradients replaced purification on glass fibers. Minipreps were made according to Birnboim and Doly (7). Total DNA from *S. lividans* was prepared as previously described (procedure 1, reference 25). Tris-acetate agarose gels, restriction endonuclease digestions, and other standard methods were as described previously (40).

Southern blot analysis. Approximately 1 µg of total DNA or 1 ng of plasmid DNA, treated with restriction endonuclease, was electrophoresed per lane in a 0.8% agarose gel for 12 to 16 h at 1.5 V/cm. Gels were depurinated, denatured, and neutralized as described previously (40) and transferred overnight in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) to Hybond-N nylon membranes (Amersham). Blots were then rinsed in 4× SSC, air dried, and UV cross-linked. Prehybridization was in Church buffer (500 mM sodium phosphate buffer [pH 7.2], 7% sodium dodecyl sulfate [SDS], 1 mM EDTA) (11) at 65°C for 60 min and was followed by hybridization overnight under the same conditions with the addition of approximately 10⁶ cpm of probe per ml. Following hybridization, filters were washed twice in 40 mM sodium phosphate buffer (pH 7.2)–1% SDS at room temperature for 15 min and, when necessary, at 65°C under the same conditions. The washed filters were exposed to

Amersham Hyperfilm-MP at room temperature or at –70°C with DuPont Cronex Lightning-Plus intensifying screens. α-³²P-labeled probes were prepared by the random primer technique with a kit purchased from Amersham and 100 ng of plasmid DNA or gel-purified restriction fragments. Radio-labeled probes were purified with Sephadex G-50 spin columns prepared in 1-ml syringes.

Plasmid constructions. Numbers in brackets refer to restriction site positions in Fig. 1. The bifunctional plasmid pOJ160 (obtained from B. E. Schoner, Eli Lilly & Co., Indianapolis, Ind.) consists of the low-copy-number basic replicon of *Streptomyces* plasmid SCP2 (5, 28, 31, 43) and the *E. coli* vector pUC19 (50), plus genes encoding resistance to thiostrepton and apramycin (which also specifies G418 resistance in both *S. lividans* and *E. coli*). The integration test plasmids pSUM48, pSUM154 (Fig. 2a), and pSUM160 were constructed by inserting the EcoRI[1]–BamHI[10], HindIII[3]–ScaI[9], and EcoRI[1]–ScaI[9] fragments, respectively, from SLP1.2 (6) (Fig. 1) into the corresponding sites of the pUC19 polylinker region of pOJ160 (the blunt *ScaI* end was inserted into the *SmaI* site). pSUM264 is a derivative of pSUM154 containing a 14-bp *XbaI* nonsense linker (CTAGTCTAGACTAG), purchased from U.S. Biochemical, Cleveland, Ohio (no. 70882), at the *SpI* site [5] that had been filled in with the Klenow fragment of DNA polymerase I.

Nucleotide sequence analysis. Clones representing approximately 150-bp sequential deletions from either end of the *intB* region DNA were obtained by exonuclease III (exoIII)-mung bean nuclease digestion with the protocol described in the Stratagene exoIII-mung bean deletion kit. Nucleotide sequence analysis (41) utilized α-³⁵S-dATP and Sequenase version 2.0 from U.S. Biochemical. Compression areas were resolved by using dITP in place of dGTP. Artifacts, presumably caused by secondary structure in the template DNA, was eliminated by addition of terminal deoxynucleotidyl transferase to the sequencing reactions just prior to their completion (19).

Computer-assisted sequence analysis. Sequence analyses utilized the software package of the University of Wisconsin Genetics Computer Group (Madison, Wis.) (17) on a VAX8550 computer. The Codon Preference program, which graphically displays the G+C composition of each base position in codons over a window (set at 25 bp), utilized a codon usage table for high G+C bias in the third base position compiled from several *Streptomyces* genes by Bibb et al. (4). The free energy of potential transcription terminators was calculated with the Genetics Computer Group program Fold, which finds the optimal secondary structure for an RNA molecule by using energies currently defined by Freier et al. (20).

Nucleotide sequence accession number. The nucleotide sequence data for this paper have been deposited in GenBank under accession number X71358.

RESULTS

A 2-kb segment of SLP1 DNA promotes integration. It was previously reported that a derivative of SLP1 that contained an insertion at the *KpnI* site [6] failed to integrate (36). To test whether DNA flanking this site could promote integration, three fragments, EcoRI[1]–BamHI[10], HindIII[3]–ScaI[9], and EcoRI[1]–ScaI[9], were subcloned into the pUC19 polylinker portion of the SCP2-based *Streptomyces* vector pOJ160, generating pSUM48, pSUM154, and pSUM160, respectively. These plasmids were introduced by

FIG. 2. Site-specific integration of pSUM154. (a) Diagram of pSUM154. The locations of the pUC19 and SCP2 replicons and genes encoding resistance to apramycin (Apr) and thiostrepton (Tsr) that are included in the bifunctional vector pOJ160 (thin line) are shown, as is the position of the 2-kb *HindIII*[3]-*ScaI*[9] fragment (thick line) from SLP1 (Fig. 1). The open squares represent *attP* and, in the integrated form, *attL* and *attR*. The predicted sizes of *BclI* (triangles)-generated restriction fragments are indicated. Hatched lines in the integrated form represent chromosomal DNA. A 1-bp difference between *attP* and *attB* generates a *BclI* site in *attB* and *attR*. (b) Southern blot analysis of total DNA from *S. lividans* TK64 digested with *BclI*. Lane 1, pOJ160 purified from *E. coli*; lane 2, TK64(pOJ160); lane 3, TK64; lanes 4 to 6, independent isolates of TK64(pSUM154); lane 7, pSUM154 purified from *E. coli*. The probe was ³²P-labeled pSUM154. Fragment sizes in kilobase pairs (kb) corresponding to autonomous pOJ160 (5.5 and 1.3 kb), the autonomous form of pSUM154 (7.3 kb), and the *attL* (6.4-kb) and *attR* (1.8-kb) fragments of integrated pSUM154 are shown.

transformation into *S. lividans* TK64, and total DNA from thiostrepton-resistant (Tsr^r) transformants was isolated, cleaved by *BclI*, and examined by Southern hybridization using ³²P-labeled pSUM154 as a probe. In each case, bands corresponding to the predicted sizes for both integrated and autonomous forms of the test plasmids were observed. A representative blot showing the integration of the smallest of these, pSUM154, is presented in Fig. 2b. As expected, parental vector pOJ160 is found only in the autonomous state (lane 2). In contrast, hybridizing signals were observed at 6.4 kb (*attL*) and 1.5 kb (*attR*), indicative of pSUM154 integration (Fig. 2b, lanes 4 to 6). In addition, a band at 7.3 kb was also observed, as predicted for the autonomous form of pSUM154 (lanes 4 and 5). Signals at 1.3 and 0.2 kb (data not shown) are common to both integrated and autonomous forms.

pOJ160, the SCP2-based vector used in these experiments, was initially developed as a transposon delivery tool and is extremely unstable (42a). Consequently, continued propagation of strains containing pSUM154 results in loss of the autonomous form (7.3 kb), leaving only integrated molecules as seen in Fig. 2b, lanes 4 and 5 (after less than one cycle of sporulation) versus lane 6 (after two cycles of sporulation).

The segment of SLP1 DNA contained on pSUM154 is also capable of promoting integration of nonreplicating DNA: insertion of the 4.5-kb *EcoRI*[1]-*BamHI*[10] fragment of SLP1 into a G418-resistant derivative of pBR322 results in

the site-specific integration of this plasmid at the *S. lividans* *attB* site (data not shown).

Nucleotide sequence of SLP1 DNA required for integration.

The nucleotide sequence (Fig. 3b) of a 2.2-kb region of SLP1 DNA beginning just upstream of the *BstBI* site [2] and extending to the *ScaI* site [9] was determined. Two likely ORFs provisionally designated *orf61* and *orf455* were identified with the Codon Preference program of the University of Wisconsin Genetics Computer Group software package. Because of the high G+C content of *Streptomyces* DNA (73 mol% G+C) (3), the third base of a codon is strongly biased toward G or C. *orf61* and *orf455* contain a third base that is G or C in 87 and 85% of codons, respectively. A summary of this region is presented in Fig. 3a.

orf61 is assumed to initiate at ATG-132 and terminate at TGA-315, generating a 61-amino-acid peptide with a predicted molecular mass of 7.1 kDa and a calculated pI of 11.04. This putative ORF is preceded by a potential ribosome binding site (GAGGAG; nucleotides [nt] 118 to 123) 9 nt upstream of the ATG. *orf455* would encode a 455-amino-acid peptide beginning at ATG-378 and terminating at TAG-1743. This ORF is preceded by a potential ribosome binding site (AGGAAGG; nt 365 to 371) 6 nt upstream of the ATG. The predicted protein would have a calculated molecular mass of 50.6 kDa and a pI of 11.13. Numerous additional ORFs larger than 150 bp were identified within this sequence; these are summarized in Fig. 3a. *orf61* and *orf455* are separated by a 61-bp region that contains a 24-bp

FIG. 3. Nucleotide sequence of the *int* region. (a) Schematic representation of the nucleotide sequence shown in panel b indicating the positions of *orf61* and *orf455*, the 112-bp *attP-attB* homology (hatched), and regions of dyad symmetry (stem-loop structures). Representative restriction sites are shown above the map; vertical lines immediately below the map correspond to 100-bp increments. Horizontal arrows indicate additional potential ORFs larger than 150 bp initiating with ATG (solid) or GTG (striped). (b) Nucleotide sequence of the 2,176-bp *int* region beginning 75 bp upstream of the *BstBI* site [2] and extending to *Scal*[9]. *orf61* initiates at nt 132 and terminates at nt 315. *orf455* (also designated *int*) initiates at nt 378 and terminates at nt 1743. Regions of dyad symmetry between *orf61* and *orf455* (nt 334 to 358) and contained within the 112-bp *attP-attB* homology (nt 1853 to 1908) are indicated by arrows. Regions of *orf61* and *orf455* showing protein sequence similarity to sequences of representative excisionases (Fig. 7) and the λ integrase family of recombinases (Fig. 6), respectively, are underlined. The thick checkered bar indicates the 112-bp *attP* region showing homology to the chromosomal DNA that contains *attB*.

segment of dyad symmetry capable of forming a stem-loop structure with a calculated $\Delta G = -17.4$ kcal (ca. -72.8 kJ) (20). A lengthy region of symmetry (nt 1853 to 1908, $\Delta G = -27.8$ kcal [ca. -116 kJ]) is also observed 108 bp downstream of *orf455*. This region lies within the 112-bp segment of homology between *attP* (nt 1910 to 1970) and chromosomal DNA containing *attB*. A similar hairpin loop structure located in an analogous region of the *Streptomyces* integrating element pSE211 (10) has been proposed to function as a transcription terminator.

Analysis of *orf455*. As described above, the SLP1 DNA segment cloned in pSUM154 promotes site-specific integration. On the basis of the nucleotide sequence, this DNA includes *orf455* and *attP*. This information allows us to tentatively assign the *orf455* gene product as an integrase and the gene encoding Orf455 as *int*.

To confirm that the product of the *int* gene is responsible for the ability of the 2-kb *HindIII*[3]-*ScaI*[9] fragment to promote integration and to support the assignment of ATG-378 as the start of this gene, we constructed a derivative of pSUM154 that contains a nonsense codon at a position corresponding to amino acid 4 of the putative Int protein. This was accomplished by inserting a 14-bp linker that contains nonsense codons in each of the three reading frames (Materials and Methods) at the *SpI* site (position 388, Fig. 3b) of pSUM154. The resulting construct, pSUM264, was introduced into *S. lividans* TK64 by transformation, and total DNA was prepared from four independent Tsr^r transformants. Southern blot analysis of *BclI*-digested total DNA (Fig. 4) using ³²P-labeled pSUM154 as a probe showed a band at 7.3 kb, corresponding to autonomously replicating pSUM264; however, in contrast to pSUM154, no bands at the positions predicted for integrated pSUM264 (6.4 and 1.8 kb) were observed. This indicates that the *int* gene is essential for integration and that sequences required for *int* activity extend across the *SpI* site, consistent with initiation of translation of *int* at ATG-378.

SLP1.2 exists in both integrated and autonomous states. SLP1.2 (6) (Fig. 1) is a naturally occurring 14.2-kb SLP1 deletion derivative previously reported to exist only in the autonomous form (36). As SLP1.2 contains the region of SLP1 shown above to be sufficient to promote stable integration, we reexamined the ability of SLP1.2 to integrate. As shown in Fig. 5, Southern blot hybridization of *BglII*-digested total DNA isolated from independent transformants of *S. lividans* C14 containing SLP1.2, with a 4.25-kb *EcoRI*[1]-*BamHI*[10] fragment (Fig. 1) as a probe, revealed a 14.2-kb band (lanes 2 and 4) predicted for the linearized autonomous form of the plasmid, as well as 12.4-kb (*attL*) and 6.0-kb (*attR*) bands corresponding to the integrated form. Similar results were obtained for pIJ13 (47), a viomycin-resistant derivative of SLP1.2. i.e., hybridization to a 16.5-kb band indicative of the linearized autonomous form and to 12.4-kb (*attL*) and 8.0-kb (*attR*) bands corresponding to the integrated form (Fig. 5, lanes 1, 3, and 5). We conclude that SLP1.2 can exist in both integrated and autonomous forms. This finding is considered further in the discussion.

DISCUSSION

The functions required for site-specific recombination of SLP1 in *S. lividans* have been localized to a 2.2-kb region of the element that maps approximately 500 bp clockwise to the unique *EcoRI* site (Fig. 1). Nucleotide sequence analysis of this region revealed two ORFs in addition to the previously identified attachment site (*attP*). On the basis of preliminary

FIG. 4. Southern blot analysis demonstrating that pSUM264 is defective in integration. The structure of pSUM264 is essentially identical to that of pSUM154 (Fig. 2a). Lane 1, pSUM154 purified from *E. coli* digested with *BclI*; lanes 2 to 5, independent isolates of TK64(pSUM264) digested with *BclI*; lane 6, TK64(pSUM154) digested with *BclI*. The sample in lane 6 showing the positions of bands corresponding to integrated pSUM154, *attL* (6.4 kb) and *attR* (1.8 kb), is from Fig. 2b, lane 6. The probe was ³²P-labeled pSUM154. Additional high-molecular-weight bands in lanes 1 and 5 result from incomplete digestion.

functional analysis of these genes as well as similarity of the derived amino acid sequences to those of known recombinases, we believe that the putative 455-amino-acid product of the *int* gene is an integrase while the product of the *orf61* gene, a putative 61-amino-acid peptide, is an excisionase (9).

The predicted amino acid sequence of the SLP1 Int protein resembles that of recombinases belonging to the λ integrase family (Fig. 6). Like other members of this group (Fig. 6; also see references 10 and 13 for examples), the SLP1 Int protein contains conserved amino acids in two regions of the C-terminal portion of the protein (domain 1, SLP1 Int positions 272 to 290, and domain 2, positions 394 to 434). Notably, SLP1 Int retains the highly conserved amino acids Arg-399 and Tyr-433 (at SLP1 positions Arg-400 and Tyr-432), as well as 11 out of 17 additional amino acids typical for this family of proteins. Tyr-433 in the bacteriophage λ integrase (37), the F1p recombinase from the *Saccharomyces cerevisiae* 2 μ m circle (22), and the Cre recombinase from bacteriophage P1 (23, 39) is known to bind covalently via a phosphotyrosyl linkage to the recombi-

FIG. 5. Integration of SLP1.2 in *S. lividans*. Southern blot hybridization to *Bgl*II-digested total DNA isolated from *S. lividans* 66 strain C14 containing pIJ13 (lanes 1, 3, and 5) or SLP1.2 (lanes 2 and 4). The probe was the 4.25-kb *Eco*RI[1]-*Bam*HI[10] fragment (Fig. 1). Fragment sizes are indicated.

tion site DNA during strand exchange. A third highly conserved amino acid, His-396, is replaced by Tyr in SLP1 Int (Fig. 6) (and by Arg in pSAM2 Int [8]). As expected from the report by Argos et al. (1), dot plot comparisons of the SLP1 Int protein sequence with those of other members of the integrase family showed the greatest degree of similarity within the carboxy-terminal region of the protein. Additional similarities were observed in comparisons of the SLP1 integrase and those of pSE211 (10) and pSAM2 (8) in the region between domains 1 and 2 (data not shown).

The product of the SLP1 *orf61* gene is predicted to be a 61-amino-acid basic peptide (7.1 kDa) that shares limited sequence similarities with Xis proteins of other site-specific recombination systems. These similarities, which span amino acids 6 to 27 (Fig. 3b), are shown aligned in Fig. 7 as a modified output from the University of Wisconsin Genetics Computer Group program Gaps. Functional analysis of this ORF, which is described in the accompanying report (9), indicates that the product of this gene both inhibits SLP1 Int-promoted integration and enhances Int-promoted excision. Consequently, we designate *orf61* as *xis*.

The *xis* and *int* genes of SLP1, like those of other site-specific recombination systems, are clustered. In several other cases, these genes are organized such that the *xis* gene lies upstream of and overlaps the *int* gene, either at the initiation-termination codon (pSAM2) (8) or more extensively (bacteriophages P22 and λ) (14, 24, 30). It has been speculated for bacteriophages λ and P22 that this organization aids in regulating the cellular concentrations of these

Domain 1

SLP1 Int 1-271	V	A	F	F	G	C	M	Y	Y	A	A	R	P	A	E	V	I	G	L	R	L	Q	D	C	-	D	L	289-396
xerC	R	A	M	L	E	V	M	Y	G	A	G	L	R	L	S	E	L	V	G	L	D	I	K	H	L	-	D	L
Tn1545 Int	Y	D	E	I	L	I	L	L	K	T	G	L	R	I	S	E	F	G	G	L	T	L	D	L	D	F	E	N
434 Int	R	L	A	M	E	L	A	V	V	T	G	Q	R	V	G	D	L	C	E	M	K	W	S	D	I	V	D	G
phi21 Int	K	C	G	M	L	L	A	L	V	T	G	Q	R	L	G	D	I	C	N	L	K	F	S	D	I	W	D	D
HK022 Int	R	L	A	M	D	L	A	V	V	T	G	Q	R	V	G	D	L	C	R	M	K	W	S	D	I	N	D	N
phi11 Int	R	Q	L	T	R	L	L	F	Y	S	G	L	R	I	G	E	A	L	A	L	Q	W	K	D	Y	-	D	K
pSAM2 Int	H	A	Y	I	V	V	A	L	L	T	G	A	R	T	E	E	L	R	A	L	T	W	D	H	V	F	L	K
Lambda Int	R	L	A	M	E	L	A	V	V	T	G	Q	R	V	G	D	L	C	E	M	K	W	S	D	I	V	D	G
P22 INT	K	S	V	V	E	F	A	L	S	T	G	L	R	R	S	N	I	I	N	L	E	W	Q	Q	I	-	D	M
P1 CRE	T	A	G	V	E	K	A	L	S	L	G	V	T	K	L	V	E	R	W	I	S	V	S	G	V	A	D	D

Domain 2

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FIG. 6. Alignment of sequences of the SLP1 Int protein to two domains present in other members of the integrase family. The regions of SLP1 Int shown are underlined in Fig. 3b. Amino acids identical or conserved are boxed. Conserved amino acids are from Dayoff et al. (15): SPAGT, RKH, FWY, DEQN, ILVM, and C. Gaps (dashes) have been introduced to maximize homology. Protein sequences were from the following sources: XerC (13), *Streptococcus pneumoniae* transposon Tn1545 (38), bacteriophage 434 (2), ϕ 21 (42), coliphage HK022 (49), *Staphylococcus* bacteriophage ϕ 11 (51), *Streptomyces* element pSAM2 (8), and a pSE211 element from *Saccharopolyspora erythraea* (10). The sequences of the Cre recombinase of bacteriophage P1 and those from bacteriophages λ and P22 were from Argos et al. (1). Additional examples are described by Brown et al. (10) and Colloms et al. (13).

SLP1	<u>M</u>	<u>L</u>	<u>K</u>	<u>L</u>	<u>S</u>	<u>E</u>	<u>V</u>	<u>L</u>	<u>E</u>	<u>E</u>	<u>I</u>	<u>E</u>	<u>M</u>	<u>S</u>	<u>R</u>	<u>A</u>	<u>A</u>	<u>F</u>	<u>Y</u>	<u>R</u>	<u>L</u>	<u>R</u>
φλ	Y	L	T	T	Q	E	W	N	A	R	Q	R	P	R	S	L	E	T	V	R	R	
P22	S	L	T	L	D	E	A	C	A	F	L	K	I	S	R	P	T	A	T	N	W	I
pSAM2	L	L	T	V	P	E	V	M	A	R	L	K	V	G	R	S	K	V	Y	N	L	I
pSE211	L	L	T	V	E	E	A	A	K	Q	L	G	L	G	R	T	K	T	Y	A	L	V
φ80	L	L	V	T	S	D	-	L	T	S	R	Y	K	I	S	R	K	T	L	W	S	Q

FIG. 7. Alignment of sequences of the SLP1 Orf61 protein to those of other known or presumed Xis proteins. The region of SLP1 Orf61 shown is underlined in Fig. 3b. Residues identical or conserved are boxed as described in the legend to Fig. 6. Protein sequences were obtained from references described in the legend to Fig. 6, with the addition of bacteriophages φ80 and P22 (30).

proteins: translation of the *xis* mRNA may sterically interfere with translation of *int* (30), thereby favoring excision by providing increased concentrations of Xis. The *xis* and *int* genes of SLP1 are organized similarly except that these genes do not overlap; rather, *xis* and *int* are separated by an intervening 61-bp region that contains a 24-bp segment of dyad symmetry ($\Delta G = -17.4$ kcal/mol [-72.8 kJ/mol]) (20) followed immediately by three A · T base pairs. Similar structures in other *Streptomyces* genes have been shown or postulated to function as transcription terminators (16, 27, 44). Attenuation of transcription initiated upstream of the SLP1 *xis* gene could provide levels of Xis and Int proteins appropriate to promote excision and guard against immediate reintegration of the excised element, as has been proposed for bacteriophage λ (18).

Our observation that pSUM154 can integrate (Fig. 2b), together with the above speculation that transcription initiated upstream of *xis* likely terminates upstream of *int*, suggests that *int* may be expressed independently of *xis*. Low-level constitutive transcription has been observed to initiate within a GC-rich dyad, similar in sequence to that observed between the *xis* and *int* genes, just upstream of the *kilB* gene on the high-copy-number *Streptomyces* plasmid pIJ101 (45) (compare the left arm of the *kilB* inverted repeat GGCCCCGCCGG with nt 337 to 350, Fig. 3b). The existence of a family of GC-rich promoters in *Streptomyces* spp. has recently been reviewed (46). Possibly, control of integrative and excisive recombination in SLP1 is mediated through low-level constitutive expression of *int* and differential expression of *xis*, as has been postulated for bacteriophages P22 and φ80 (30). Potentially, this regulatory strategy could provide a pool of Int capable of reintegrating elements excised by spontaneous excision or homologous recombination of *attL* and *attR*, consistent with the apparent stability of the SLP1 element in the *S. coelicolor* chromosome.

The results reported here show that together *int* and *attP* can promote SLP1 integration in *S. lividans*. A second region of SLP1 previously had been implicated as functioning in site-specific recombination (36). The existence of this locus, *intA* (Fig. 1), was postulated to explain the observation that the naturally occurring SLP1 deletion derivative SLP1.2 was found only as an autonomously replicating plasmid despite the presence of DNA believed capable of mediating integration (*intB*, shown here to consist of *xis*, *int*, and *attP*). When DNA that spanned the region deleted in SLP1.2 (*intA*; see Fig. 1) was cloned and provided in *trans*, SLP1.2 was found only in the integrated form (36). We now find that SLP1.2 can in fact exist in both integrated and autonomous forms (Fig. 5). The finding that the *intA* locus in *trans* resulted in loss of autonomous SLP1.2 plasmids parallels recent findings by Grant et al. (21) that a function

encoded by SLP1, designated *imp*, inhibits the maintenance of the autonomous form of SLP1 and may silence replication of the integrated SLP1 element; both *intA* and *imp* map to the same region of SLP1 (Fig. 1).

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