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Replicase, Excisionase, and Integrase Genes of the *Streptomyces* Element pSAM2 Constitute an Operon Positively Regulated by the *pra* Gene

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pSAM2 is a site-specific integrative element from *Streptomyces ambofaciens*. The *pra* gene described earlier as an activator of pSAM2 replication is shown here to be also involved in the activation of its integration and excision. This was evidenced with derivatives of pSAM2 mutant B3 in which the *pra* gene was placed under the control of the inducible *tipAp* promoter. Transformation of *Streptomyces lividans* by these derivatives was efficient only when *pra* expression was induced, indicating its involvement in pSAM2 integration activation. Once established, these constructions remained integrated in the chromosome under noninduced conditions. Activation of the *pra* expression provoked strong activation of their excision, leading to the appearance of free forms. The results of functional, transcriptional, and sequence analyses allowed to conclude that the three genes *repSA*, *xis*, and *int* coding for the pSAM2 replicase, excisionase, and integrase, respectively, constitute an operon directly or indirectly activated by *pra*.

The integrative elements of *Actinomycetes* are a special class of mobile genetic elements, found only in this group of bacteria, characterized by their ability to integrate in the host chromosome by recombination between the chromosomal attachment site *attB* and the element site *attP*. Like plasmids, integrative elements are able to transfer and replicate and their free and integrated forms may coexist (reference 21 and references therein). However, unlike plasmids, for integrative elements replication is not essential for maintenance but for the propagation of the element during conjugation.

pSAM2 is an 11-kb element originally isolated in *Streptomyces ambofaciens*, which produces the macrolide antibiotic spiramycin (15). pSAM2 can replicate (7, 8), is self-transmissible (9), elicits the lethal zygosis reaction (pock formation), and mobilizes chromosomal markers (25). pSAM2 also has a site-specific recombination system very similar to that of temperate bacteriophages (4–6). The product of the *int* gene promotes site-specific integration, and the product of the *xis* gene, together with Int, promotes excision of pSAM2 (18). After integration, pSAM2 is stably maintained integrated in the chromosome during the entire host life cycle of growth and differentiation.

pSAM2 replicates via a rolling-circle mechanism. The *repSA* gene coding for the replication initiator protein and the *ori*$^+$ sequence involved in the initiation of replication have been characterized (7, 8). The study of the replication control led to the discovery of *pra*, a positively acting regulator (21). It was demonstrated that *pra* was indispensable for pSAM2 replication but was not directly involved in the machinery of replication. When the *pra* gene, carried by a multicopy vector, was transcribed from the constitutive and strong *ermE** promoter (1, 2), it could confer in *trans* to $pSAM2_{B2}$, which is normally observed integrated, the capacity to exist integrated and free, a phenotype normally characteristic of $pSAM2_{B3}$ (21).

In a previous study (7), a DNA sequence analysis, it was suggested that the three genes *repSA*, *xis*, and *int* could form an operon. In this study, we demonstrated that *pra* acts as a positive regulator not only for pSAM2 replication but also for integration and excision and we confirmed by functional and transcriptional analyses that *repSA*, *xis*, and *int* are organized as an operon.

MATERIALS AND METHODS

Bacterial strains, growth, and transformation. *Streptomyces lividans* TK24 (12) was used as the host strain. General culture conditions and genetic techniques for *Streptomyces* spp. and for *Escherichia coli* were as described by Hopwood et al. (11) and Sambrook et al. (19), respectively.

Streptomyces transformants carrying the thiostrepton resistance (*tsr*) gene (29) were selected with 50 μg of nosiheptide ml^{-1}. Transformants carrying the hygromycin resistance gene were selected with 200 μg of hygromycin B (Boehringer Mannheim) ml^{-1} in R2YE medium, and then they were maintained in HT medium (16) with 50 μg of hygromycin ml^{-1}. The inductive dose of nosiheptide was 0.1 μg ml^{-1}.

Construction of pOS546, pOS548, and pOS693. To construct pOS548, the 2.0-kb *Asp*718I-*Asp*718I fragment of pTS39 (Table 1) was replaced by the 2.4-kb *Asp*718I fragment that differs from the initial fragment only by the replacement of the *pra* gene promoter by *tipAp* (10, 14). The fd terminator was placed upstream of *tipAp*. pTS39 codes for all the functions characterized in pSAM2 (replication, integration, transfer, pock formation, and mobilization of chromosomal markers), and it possesses the *tsr* resistance gene. The hygromycin resistance gene *hyg* (30) was introduced in the unique *Hin*dIII site as a second selective marker during pOS548 construction. pOS546 was constructed as pOS548 was, except pTS74 was used, instead of pTS39. pTS74 is a pTS39 derivative with the *repSA* gene inactivated by filling in the *Bcl*I(18533) site (the number in parentheses refers to the nucleotide position of the site in Fig. 1).

To construct pOS693, the *Eco*RI(15493)-*Eco*RI(19700) fragment from pOS548 was replaced by the same fragment containing the Ω*aac* cassette (3) inserted into the *Apa*I(18514) site in the *repSA* gene.

Status of pSAM2 derivatives in *S. lividans*. For Southern hybridizations, the probe was labelled by using the ^{T7}QuickPrime kit from Pharmacia LKB. With labelled oligonucleotide, hybridization was performed at 55°C in a solution containing 0.5 M NaH_2PO_4/Na_2HPO_4 buffer (pH 7.2), 7% sodium dodecyl sulfate, 1% bovine serum albumin, and 1 mM EDTA, and filters were washed at 55°C in

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TABLE 1. Plasmids used in this study

Plasmid	Construction[a]	Status of pSAM2 derivatives in *S. lividans*[b]	Reference
pOS546	pOS548 derivative constructed with pTS74 (see Materials and Methods)	Status after establishing: INT without *tipAp* induction; free and INT with *tipAp* induction	This work
pOS548	pTS39 derivative in which the *pra* gene promoter was replaced by *tipAp* (see Materials and Methods)	Status after establishing: INT without *tipAp* induction; REP and INT with *tipAp* induction	This work
pOS549	pOS546 derivative with the *pra* gene disrupted by filling of the *Asp*718I(3302) site	NT in *S. lividans*; free and INT in *S. lividans*/pOS689	This work
pOS550	pOS548 derivative with the 3′ part of the *pra* gene deleted (2-kb deletion in the *pra-traSA-spdA* region)	NT in *S. lividans*	This work
pOS693	pOS548 derivative with the *repSA* gene disrupted by insertion of Ω*aac* (3) in the *Apa*I(18514) site	NT in *S. lividans*	This work
pOS11Δ	Deletion variant of pOS11 (22); high copy number in *S. lividans*	REP and INT	24
pTO1*	TO1 derivative which does not contain the *tipA* promoter; integrative vector containing pBR322, a fragment of phage ϕC31 with its *attP* site and *int* gene for integration in *Streptomyces* spp.		28 This work
pOS689	*Bgl*II fragment from pOS541 (21) containing *ermE*p*-RBS-*pra* cloned in the *Bam*HI site of pTO1*		This work

[a] *tipAp* is the inducible *tipA* promoter, and *ermE*p* is the constitutive *ermE** promoter. The numbers after some restriction sites correspond to the positions of the sites in Fig. 1. RBS, ribosome binding site.
[b] INT, integrative; REP, replicative; NT, no transformants.

a solution of 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate.

RNA isolation, Northern hybridization, and high-resolution S1 mapping. Total RNA from *S. lividans* was isolated by the method of Hopwood et al. (11). For Northern hybridization, total RNA (40 to 50 μg) was denatured with glyoxal and dimethyl sulfoxide (19), subjected to electrophoresis, and then transferred to Hybond-N filter (Amersham).

High-resolution S1 mapping was performed by the method of Hopwood et al. (11). The probe was prepared by the method of Raibaud et al. (17), using the oligonucleotide AMD3 that is situated 41 bp downstream of the presumed start codon of the *repSA* gene (see Fig. 5). The sequence used to determined the sizes of the protected fragments was obtained with the *Bcl*I(18533)-*Eco*RI(19700) pSAM2 fragment (Fig. 1) cloned in M13mp18 and sequenced with the standard M13 −40 primer.

Nucleotide sequence accession number. All published parts of pSAM2 sequence (8,820 bp) (5–9, 21) were assembled and are now available in the EMBL data bank under accession no. AJ005260.

RESULTS

The *pra* gene is required for efficient pSAM2 integration. Previous results (21) allowed us to conclude that the *pra* gene codes for an activator of pSAM2 replication. In order to mimic the role of Pra in the regulation of other pSAM2 functions, it was expressed in *cis* from an inducible heterologous *tipA* promoter (*tipAp*) in the context of the complete pSAM2 sequence (a derivative named pOS548 [Fig. 1]).

Transformation of *S. lividans* by pOS548 gave a surprising result. The transformation was efficient (5×10^3 clones per μg of DNA) only under inducing conditions, and only a few colonies were observed in the absence of the inducer nosiheptide. To eliminate the possible effect of replication on transformation efficiency, similar experiments were performed with a nonreplicating derivative of pOS548, pOS546, in which the *repSA* gene was inactivated (Table 1). In this case, transformation efficiency is a direct reflection of integration efficiency. As for pOS548, transformation of *S. lividans* with pOS546 was efficient only under inducing conditions.

The integrity of *pra* gene is necessary, as null mutants (pOS549 and pOS550) were unable to transform *S. lividans* TK24 efficiently whether the expression of *ptipA* was induced or not.

These results indicate that *pra* is required for the efficient integration of pSAM2. However, it does not code for a protein directly involved in pSAM2 site-specific-integration, as integrative derivatives not containing the *pra* gene could transform *S. lividans*. For instance, pTS33 (23) and pOS551 (20), in which the expression of the *int* gene is not under its normal control, had high transformation efficiencies. The role of Pra as an integration activator was confirmed with *S. lividans*/pOS689, where *pra* is constitutively expressed in *trans*. Unlike the situation with the wild type, it was possible to transform this species efficiently with pOS546, pOS548, pOS549, and pOS550, even in the absence of the inducer. It should be noted that all the integrations observed with the pSAM2 derivatives occurred through site-specific integration at the chromosomal pSAM2 *attB* site (Fig. 2 and 3).

Pra could activate pSAM2 excision. Transformants obtained with pOS548 and pOS546 in the presence of the inducer were studied to determine the status of pSAM2 derivatives (integrated or free). Southern hybridization allowed us to demonstrate that pOS546 and pOS548 integrate in the chromosome of *S. lividans* site specifically (Fig. 2 and 3).

Under noninduced conditions, only the integrated copy was detected for both constructions (Fig. 2A, lane 1; Fig. 3A, lanes 3 and 4). For pOS548, in the presence of a very low concentration of the inducer (0.01 μg/ml), a 4.2-kb band appeared, indicating the presence of its free form (Fig. 2A, lane 2). In the presence of a higher concentration of the inducer, the excision and replication of pOS548 were strongly activated (Fig. 2A, lanes 3 and 4). This was confirmed also by detection in these DNAs of a high proportion of unoccupied chromosomal *attB* sites (Fig. 2B).

pOS546, a pOS548 derivative in which *repSA* is inactivated, could be a better model to study the excision, as it could not replicate. Excision was never observed with pTS74, a pOS546 precursor with *pra* expressed from its own promoter (data not shown). Induction of the *pra* gene expression led to activation of pOS546 excision (Fig. 3A, lanes 1 and 2). The appearance of the free form of pOS546 was accompanied by the appearance of nonoccupied chromosomal *attB* sites (Fig. 3B). It demonstrates that Pra activates pSAM2 excision even in the absence of replication and not through activation of replication.

To demonstrate that Pra is necessary for excision, we used a derivative of pOS546, pOS549, in which the *pra* gene was inactivated. The DNA obtained from rare transformants of *S. lividans*/pOS549 was analyzed by Southern hybridization. In all cases, pOS549 was site specifically integrated, but in *S. livi-*

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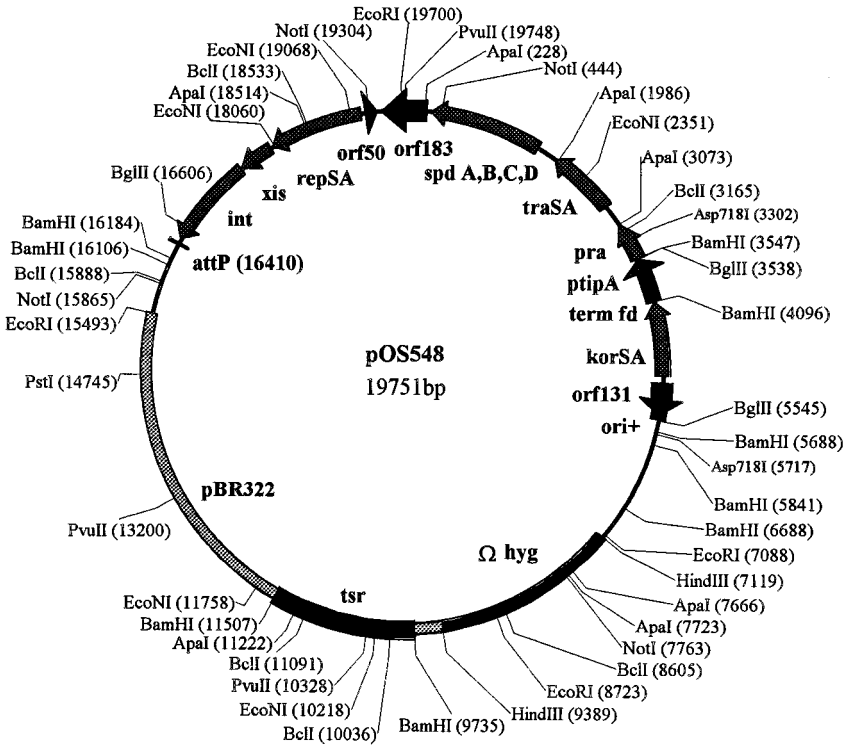

FIG. 1. Map of pOS548 showing the pSAM2 genes and open reading frames and the *attP* and *ori*$^+$ sites. The *pra* gene promoter was replaced by the fragment containing the phage fd transcriptional terminator (term fd) and the *tipA* promoter (*ptipA*) inducible by nosiheptide. The two resistance genes, *tsr* (conferring resistance to nosiheptide [29]) and *hyg* (conferring resistance to hygromycin [30]) are shown. The pBR322 replicon allows the maintenance of pOS548 in *E. coli* and carries an ampicillin resistance gene. The number in parentheses refers to the nucleotide position of the site.

dans/pOS549, excision of pOS549 could not be obtained even under inducing conditions (data not shown). This was due to the absence of *pra*, because excised forms of pOS546, pOS548, and pOS549 were detected in *S. lividans*/pOS689 (in which another copy of *pra* was expressed in *trans*) in the presence or absence of the inducer (Fig. 2A, lanes 5, 6, 7, and 8; Fig. 3A, lanes 5 and 6).

These results allowed us to conclude that in addition to its

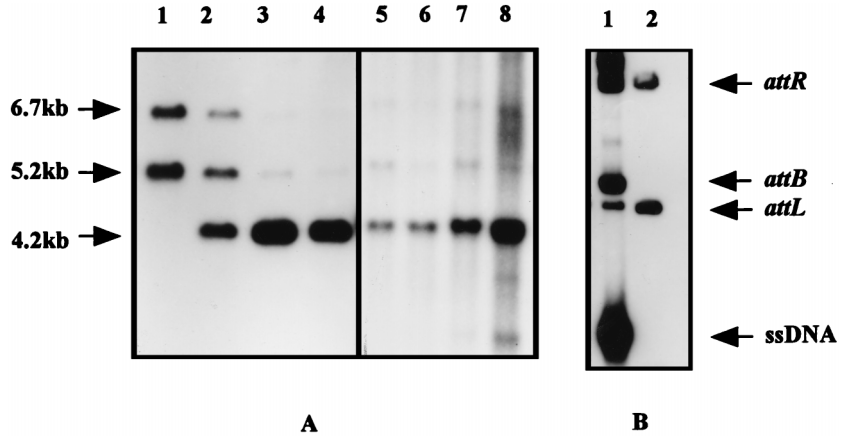

FIG. 2. Effect of *pra* gene expression on the status of pOS548 in *S. lividans* and *S. lividans*/pOS689. (A) Appearance of the free form of pOS548. Total DNA digested by *Eco*RI was analyzed by Southern hybridization with the ^{32}P-labelled *Eco*RI(15493)-*Eco*RI(19700) pSAM2 fragment (Fig. 1). Total DNA was extracted from *S. lividans* (lanes 1 to 4) and from *S. lividans*/pOS689 (lanes 5 to 8) containing pOS548 and grown in the presence or absence of nosiheptide as *tipAp* inducer. The 6.7- and 5.2-kb fragments indicate the presence of pOS548 integrated at the *attB* site. The 4.2-kb fragment indicates the presence of the free form of pOS548. Lane 1, no nosiheptide; lane 2, 0.01 μg of nosiheptide ml^{-1}; lane 3, 0.1 μg of nosiheptide ml^{-1}; lane 4, 1.0 μg of nosiheptide ml^{-1}; lane 5, no nosiheptide, clone 1; lane 6, no nosiheptide, clone 2; lane 7, 0.1 μg of nosiheptide ml^{-1}, clone 1; lane 8, 0.1 μg of nosiheptide ml^{-1}, clone 2. (B) Appearance of unoccupied *attB* sites. Total DNA from *S. lividans*/pOS548 digested by *Pst*I was analyzed by Southern hybridization with the ^{32}P-labelled 40-mer oligonucleotide probe OL-1 that corresponds to a part of the identity segment between the *S. lividans attB* and the pSAM2 (and pOS548) *attP* sites (6). Total DNA was extracted from *S. lividans*/pOS548 grown in the presence (lane 1) or absence (lane 2) of inducer. The positions of *attB*, *attR*, and *attL* are indicated by arrows. The unoccupied *attB* site is situated in a 7.5-kb chromosomal *Pst*I DNA fragment. If the *attB* site was occupied by pOS548, fragments of 6.3 and 21.0 kb containing the *attL* and *attR* sites, respectively, were detected. *attP* and *attR* are carried by fragments of 19.75 and 21.0 kb, respectively, that were not resolved in the gel. The position of ssDNA is also indicated.

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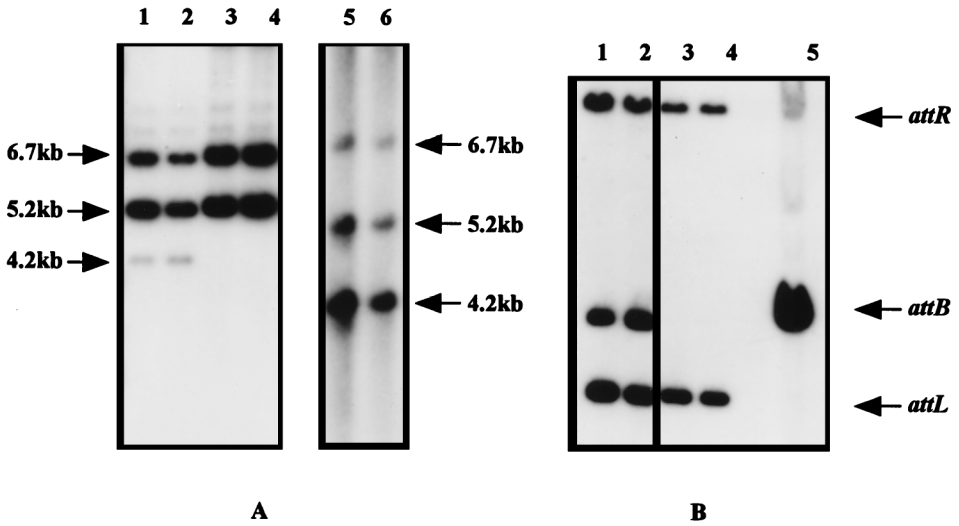

FIG. 3. Effect of *pra* gene expression on the status of pOS546 in *S. lividans*. (A) Appearance of the free form of pOS546. Total DNA digested by *Eco*RI was analyzed by Southern hybridization with the ^{32}P-labelled *Eco*RI(15493)-*Eco*RI(19700) pSAM2 fragment (Fig. 1). The total DNA was extracted from *S. lividans* (lanes 1 to 4) and from *S. lividans*/pOS689 (lanes 5 and 6) containing pOS546 and grown in the presence or absence of inducer. The 6.7- and 5.2-kb fragments correspond to pOS546 integrated at the *attB* site. The 4.2-kb fragment corresponds to the free form of pOS546. Lane 1, 0.1 μg of nosiheptide ml^{-1}, clone 1; lane 2, 0.1 μg of nosiheptide ml^{-1}, clone 2; lane 3, no nosiheptide, clone 1; lane 4, no nosiheptide; lane 5, no nosiheptide, clone 1; lane 6, 0.1 μg of nosiheptide ml^{-1}, clone 1. (B) Appearance of the unoccupied *attB* site. Total DNA from *S. lividans*/pOS546 digested by *Pst*I was analyzed by Southern hybridization with the ^{32}P-labelled 40-mer oligonucleotide probe OL-1. Total DNA was extracted from *S. lividans*/pOS546 grown as follows. Lane 1, with inducer, clone 1; lane 2, with inducer, clone 2; lane 3, no inducer, clone 1; lane 4, no inducer, clone 2; lane 5, *S. lividans* TK24 (no plasmid). For details, see the legend to Fig. 2.

already defined activation role for replication and integration Pra could also activate pSAM2 excision.

The *repSA*, *xis*, and *int* genes constitute an operon with two transcriptional start points. The ability of Pra to activate three functions (replication, integration, and excision) and the genetic organization of the *repSA*, *xis*, and *int* genes suggest that they could be cotranscribed. It was observed previously that the *repSA* stop codon overlapped the start codon of the *xis* gene and the *xis* stop codon overlapped the *int* start codon. The only inverted repeats that could constitute a rho-independent transcriptional terminator were found downstream of the *int* gene. Functional analysis results were also consistent with this hypothesis (5, 7). The replicative pSAM2 derivative pOS548 was used to confirm these results.

The Ω*aac* cassette (3) was used to introduce translation and transcription stop signals in the *repSA* gene, yielding derivative pOS693. This derivative was unable to integrate into the chromosome (no transformants were obtained) under induced or noninduced conditions for *pra* expression. This could be explained only by interruption of transcription of the downstream situated *int* gene, as a nonpolar disruption of the *repSA* gene in pTS74 and pOS546 did not abolish integration.

To directly demonstrate the operon organization of the *repSA-xis-int* genes, analysis of the size of the corresponding mRNA transcript was performed. Northern hybridization was done with total RNA isolated from *S. lividans*/pSAM2$_{B3}$ in which *pra* is constitutively expressed and for which the replicative and integrated forms coexist. A DNA fragment carrying the *repSA* gene was used as a probe. The presence of a highly unstable transcript was revealed. It gave a pattern of degraded RNA with some poorly visible diffused bands starting from a level corresponding to a size of about 4 to 5 kb (data not shown). The same results were obtained with other probes corresponding to the *repSA-xis-int* DNA fragment and by low-resolution S1 mapping (data not shown). The degradation was specific for mRNA hybridizing with *repSA*, as rehybridization of the same Northern filter with a DNA probe corresponding to the *korSA* genes revealed a single nondegraded band with the size expected for the *korSA* transcript (data not shown). Together with the results of DNA and functional analyses, these results allowed us to conclude that the three genes *repSA*, *xis*, and *int* form an operon that code for a highly unstable mRNA.

To localize the promoter(s) of the *repSA-xis-int* operon, the position(s) of its transcriptional start point(s) was determined by high-resolution S1 mapping. As shown on Fig. 4, one major and one minor transcriptional start point were revealed. In *S. lividans*/pSAM2$_{B3}$ total RNA, the detected fragments migrated as bands 220 and 232 nucleotides (nt) long. The strongest band was the 232-nt fragment. Upstream of these transcriptional start points, there is no sequence similar to the consensus sequences for −35 and −10 regions (27).

DISCUSSION

The analysis of the pSAM2$_{B3}$ for which the free and integrated forms coexist led to the identification and characterization of the *pra* gene (7) that was proposed to be an activator of pSAM2 replication (21). To analyze further its role, it was decided to construct a plasmid with the *pra* gene in *cis*, expressed under the control of a heterologous inducible promoter in order to compare the expression of *int*, *xis*, and *repSA* under conditions of expression or nonexpression of *pra*.

The replicative and integrative derivative pOS548 and the integrative derivative pOS546, in which the *repSA* gene is inactivated, transform *S. lividans* with a very low efficiency if the expression of *pra* is not induced. Once integrated, they did not excise in the absence of induction, as revealed by the absence of the free forms and of free *attB* sites. For the nonreplicative pOS546, the efficiency of transformation directly reflects the efficiency of integration. Low efficiency of transformation by site-specific integration, observed for these plasmids under noninduced conditions, but also for other *pra*$^-$ pSAM2 derivatives, could likely be due to a poor basal expression of the *int* gene. When *pra* was induced, the efficiency of transformation

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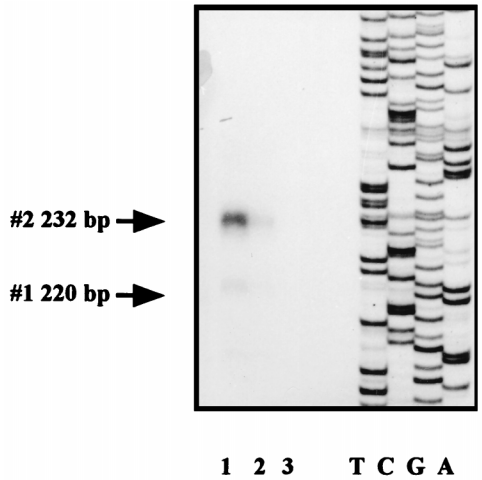

FIG. 4. High-resolution S1 mapping of the *repSA* gene transcriptional start. To obtain a ssDNA corresponding to the presumed promoter region of the *repSA* gene, the 1.16-kb *Bcl*I(18533)-*Eco*RI(19700) (Fig. 1) fragment of pSAM2 was cloned in the *Bam*HI and *Eco*RI sites of the M13mp18 vector and its ssDNA was isolated. To synthesize the second strand, this ssDNA was annealed with labelled oligonucleotide AMD3. Synthesized double-stranded DNA was directly digested with *Eco*RI, giving a labelled fragment of 537 bp. Total RNA was hybridized with this DNA fragment and treated with S1 nuclease. Lane 1, DNA probe treated with S1 enzyme in the presence of total RNA from *S. lividans*/pSAM2$_{B3}$; lane 2, DNA probe treated with S1 enzyme in the presence of total RNA from *S. lividans*/pOS11Δ; lane 3, DNA probe treated with S1 enzyme in the absence of total RNA. The sizes of the revealed protected DNA fragments were determined with the sequence of the *Bcl*I(18533)-*Eco*RI(19700) fragment (Fig. 1; fragment 6641 to 5477 in the EMBL sequence) cloned in M13mp18 and sequenced with the standard −40 oligonucleotide.

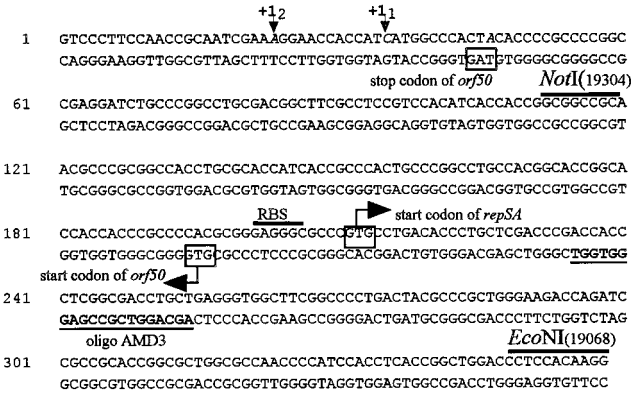

FIG. 5. Transcriptional start points of the *repSA* gene. The sequence upstream and downstream of the *repSA* gene start codon is presented (positions 5761 to 6118 in the EMBL sequence). The positions of the two transcriptional start points are indicated by +1, followed by the number corresponding to the signal numbers on Fig. 4 and also marked by vertical arrows. The positions of the presumed start and stop translation codons and the restriction sites *Eco*RI and *Not*I are indicated. The numbers in parentheses correspond to their positions in Fig. 1. RBS, ribosome binding site; oligo, oligonucleotide.

was high and the transformants contained the free form, in addition to the integrated one, indicating excision and replication for pOS548 and excision for the nonreplicative pOS546 (presence of free *attB* sites). In the case of pOS548, single-stranded DNA (ssDNA), an intermediate in the replication by a rolling-circle mechanism, was detected, as expected, if Pra activates replication. To prove that this positive regulation was not due to a transcription of the *int* and *xis* genes from the strong *tipAp* promoter situated far upstream, the *pra* gene was disrupted and it was no longer possible to activate integration, excision, and replication. However, this activation could be restored if *pra*, cloned in an integrative monocopy vector, was expressed constitutively in *trans.*

It should be stressed that induction of *pra* in *cis* led to a strong activation of pOS548 and pOS546 excision, as judged by the appearance of nonoccupied chromosomal *attB* sites. It is different from the results observed with *pra* constitutively expressed from its promoter in *cis* in the mutant pSAM2$_{B3}$ or expressed in *trans* from the *ermE** promoter (21). In these cases, replication was activated without the appearance of the free *attB* sites. In explaining this difference, the influence of introducing a strong heterologous promoter (*tipAp*) upstream of *pra* cannot be excluded. It could change the transcription in the downstream situated *traSA-spd* region where some genes could be also involved in the regulation of pSAM2 functions (unpublished observation). However, the results obtained with pOS689/pOS548, pOS689/pOS546, pOS549, and pOS550 directly demonstrated a predominant role of *pra* in the activation of pSAM2 excision. Pra activates the integration and excision independently of replication, as was demonstrated for the nonreplicative variant pOS546 where *repSA* was inactivated by a nonpolar mutation. However, the polar mutation introduced in *repSA* (pOS693) abolished activation of integration by Pra, as was deduced from the absence of transformants.

These results broadened the role of *pra* and strongly suggest that it directly or indirectly activates transcription of the *repSA*, *xis*, and *int* genes. The results of functional and transcriptional analyses of the *repSA*, *xis*, and *int* genes suggest that they form an operon. The transcript is unstable, but it was nevertheless possible to determine its transcription start points. The locations of these start points are in agreement with the results of functional analysis (7), suggesting that transcription began between the *Sma*I(19427) and *Not*I(19304) sites (Fig. 5). These data allowed us to conclude that the *repSA-xis-int* transcript covers the region containing *orf50* (7) situated upstream of *repSA* and read in the opposite direction. *orf50* has a typical *Streptomyces* codon usage. *orf50* could be involved in pSAM2 replication (as the minimal replicon of pSAM2 contains *orf50*) and/or its regulation.

Replicative and integrative vectors constructed on the basis of pSAM2$_{B3}$ constituted a powerful tool for cloning in *Streptomyces* (13, 20, 26). Identification and study of *pra*, which regulates several key pSAM2 functions, could open a way to build a new generation of pSAM2-based vectors. The replicative derivative pOS548 can exist as one integrated copy without induction, when *pra* is not expressed, and in several free copies per genome when it is expressed after induction. Identification of additional elements regulating pSAM2 should aid in constructing new vectors. These vectors could be used to express genes coding for toxic products or at a specific step of the culture.

While integration of pSAM2 is provided by Int alone, excision needs the simultaneous presence of Int and Xis. This implies, in addition to the regulation of the transcription of the *rep-xis-int* operon by *pra*, an additional modulation of the respective activities of Rep, Xis, and Int to ensure integration, excision, and replication of pSAM2.

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