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Promoter Determining the Timing and Spatial Localization of Transcription of a Cloned *Streptomyces coelicolor* Gene Encoding a Spore-Associated Polypeptide

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Streptomyces coelicolor **is a filamentous, gram-positive bacterium that exhibits a complex cycle of morphological differentiation involving the formation of an aerial mycelium of multinucleoid hyphae which undergo septation to form long chains of spores. We report the identification of two proteins of 13 and 3 kilodaltons, designated SapA and SapB, respectively, that are produced during formation of the aerial mycelium and are found in association with purified, mature spores. We cloned the structural gene (*sapA*) for one of these spore-associated proteins. Nucleotide sequence analysis suggests that the 13-kilodalton polypeptide is derived from a larger pre- or preproprotein containing a leader sequence of 37 amino acids. Nuclease protection-hybridization analysis and experiments using the *Vibrio harveyi*, luciferase-encoding *luxAB* operon as a gene tag demonstrated that expression of *sapA* is controlled from a promoter contained within a region of less than 110 base pairs in length, whose transcription start site is located approximately 50 base pairs upstream from the initiation codon for the *sapA* open reading frame. Transcription of *sapA* was induced at the time of appearance of the aerial mycelium, and the level of *sapA* transcripts was significantly reduced in certain mutants blocked in aerial mycelium (*bld*) and or spore (*whi*) formation. As further evidence of the association of *sapA* transcription with morphological differentiation, experiments in which we monitored *sapA* transcription topographically by use of a *sapA-luxAB* operon fusion demonstrated a close spatial correlation between colony regions undergoing aerial mycelium formation and zones of *sapA*-promoted light emission.**

Members of the genus *Streptomyces* are gram-positive soil bacteria that grow through the formation of long multinucleoid hyphae, which branch repeatedly as they elongate to form a substrate mycelium (for a review, see reference 3). During growth on a solid substrate, the colonies undergo a complex cycle of morphological differentiation. As nutrients become limiting for growth, hyphae begin to project from the surface of the substrate mycelium to form a morphologically distinct aerial mycelium. Mutants unable to form the aerial mycelium produce smooth colonies that are referred to as having a "bald" phenotype. Aerial filaments change into chains of spores by the formation of regularly spaced cross walls that partition the hyphae into single-nucleoid compartments. After septation is complete, the compartments undergo metamorphosis into spores, which are initially held together in long chains but eventually separate from each other to give rise to single spores. Mutants in which the aerial mycelium is unable to form spores are recognized by their characteristic "white" phenotype (in most species, the wild-type turns grey or other colors as spores are formed).

An advantageous system for studies of morphological differentiation in *Streptomyces* is the species *Streptomyces coelicolor*, for which well-developed tools of traditional and molecular genetics are available (3, 7). More than a dozen genes referred to as *bld* (mutations in which cause the bald phenotype) and *whi* (mutations in which cause the white phenotype) that are required for normal spore formation have been identified by mutation and mapped on the *S. coelicolor* chromosome (2, 14), and three of these have been cloned and manipulated in ways that have provided insights into how their gene products are involved in differentiation (13, 19; K. Chater, personal communication). As a complementary approach to studying spore formation in *S. coelicolor*, we have sought to identify spore-associated proteins whose time of appearance is developmentally regulated. Here we report the identification of spore-associated proteins of 13 and 3 kilodaltons (kDa), the application of a reverse genetic strategy to the cloning of the structural gene for one of these proteins, and, finally, the use of the cloned gene in studying temporal and spatial aspects of its regulation during spore formation.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Escherichia coli* JM83 and JM101 (25) were used for cloning into plasmid pUC18 and bacteriophages M13mp18 and M13mp19, respectively. Bacterial transformations were performed as described by Maniatis et al. (11), and the strains were grown in 2YT or LB media (15). *S. coelicolor* A3(2) was used for protein and DNA purifications. Media and procedures described by Hopwood et al. (8) were used for growth of *S. coelicolor* and total DNA isolation. *S. coelicolor* developmental mutants have been described previously (2, 7, 14).

Mycelia and spores. *S. coelicolor* cells were grown at 30°C on nitrocellulose membrane filters (HATF08225; Millipore Corp.) placed on R2YE agar medium. Mycelia were scraped off the surface of the plates by using a bent spatula. The mycelia were suspended in 1 M KCl solution and then washed twice with distilled water and harvested by centrifugation at 10,000 × *g* for 10 min at 4°C. Spores harvested

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from 7-day cultures were purified by filtration through cotton wool two times, followed by centrifugation at 10,000 × *g* for 10 min at 4°C.

Protein extraction. Surface protein was extracted by a modification of the method of Goldman and Tipper (6). Mycelia and spores were suspended in 50 mM sodium carbonate-bicarbonate buffer (pH 10) containing 1% (wt/vol) sodium dodecyl sulfate (SDS), 50 mM dithiothreitol (DTT), and 2 mM phenylmethylsulfonyl fluoride (PMSF) and incubated at 37°C for 90 min with occasional vortexing. After centrifugation twice at 10,000 × *g* for 10 min at room temperature, trichloroacetic acid was added to the supernatant to a final concentration of 12% (vol/vol). Next, the supernatant fluid was incubated on ice for 30 min, and the resulting precipitate was collected by centrifugation at 10,000 × *g* for 10 min at 4°C, washed with 5% trichloroacetic acid, and collected by centrifugation again. Extracted protein was characterized by high-resolution SDS-polyacrylamide gel electrophoresis, using 18% polyacrylamide (23).

Purification of spore-associated proteins and sequencing of SapA. The SapA and SapB proteins were purified by electrophoretic elution from 1.5-mm-thick preparative polyacrylamide gel slices containing the purified polypeptides as described by Hunkapeller et al. (9). After ethanol precipitation, the proteins were subjected to automated Edman degradation in an Applied Biosystems Gas-phase Sequenator (model 470A) and analyzed on a Dupont Zorbax C18 column in a Hewlett-Packard high-pressure liquid chromatograph (model 1090) with a 1040 diode array detector. This analysis was carried out by the Microchemistry Facility of the Harvard Biological Laboratories.

Synthetic oligonucleotide. A 51-mer oligonucleotide of the sequence 5′-T-A-C-A-A-C-G-G-C-G-T-C-A-C-C-G-G-C-T-C-C-G-G-C-T-A-C-A-A-C-G-T-C-G-T-C-A-A-C-T-C-C-A-T-G-C-C-G-A-T-C-3′ was synthesized in an Applied Biosystems DNA synthesizer (model 380A) in the Microchemistry Facility in the Harvard Biological Laboratories. Purification and end labeling were carried out according to the method of Binnie et al. (1).

Cloning. To identify endonuclease restriction fragments of *S. coelicolor* DNA carrying the *sapA* coding sequence, 20 μg of chromosomal DNA was digested with one or several endonucleases and subjected to electrophoresis in a 0.8% agarose gel containing Tris-acetate electrode buffer (11). The DNA was transferred to Millipore nitrocellulose, and hybridizations were carried out according to the method of Donovan et al. (5), except that 6× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) was used and the hybridization was carried out at 60°C. The filters were successively washed at incrementally higher temperatures to 73°C. For cloning, we identified a unique 1.5-kilobase *Bam*HI-*Sal*I DNA fragment which hybridized strongly with the 51-mer.

Recombinant pUC18 plasmids containing size-selected *S. coelicolor* *Bam*HI-*Sal*I restriction fragments were introduced into *E. coli* JM83 by transformation. Ampicillin-resistant transformants were then grown on nitrocellulose filters for use in colony hybridization experiments with radioactively labeled oligonucleotide.

DNA sequencing. The nucleotide sequence of both DNA strands of part of the *sapA* coding sequence was determined by the chain termination method of Sanger et al. (21). The *Nar*I-*Nar*I fragment of 498 base pairs (bp) was subcloned into the phages M13mp18 and M13mp19 for sequencing analysis. To eliminate several ambiguities in the sequencing analysis, we cloned the two *Sau*3AI fragments from the *Nar*I-*Nar*I DNA into the phage vectors and subjected the subcloned DNAs to sequencing analysis, substituting 7-deaza-dGTP (17) or dITP (16) for dGTP.

RNA purification. *S. coelicolor* cells were grown on nitrocellulose filters on R2YE plates and scraped at appropriate times during growth. The mycelia were suspended in chilled distilled water and collected by filtration on Whatman no. 1 disks or by centrifugation at 10,000 × *g* for 10 min. RNA was prepared from each sample by the procedure of Penn et al. (18) as modified by Igo and Losick (10). Contaminating DNA in the RNA preparations was removed by digestion with RNase-free DNase (Promega Biotec, Madison, Wis.). The RNAs were undegraded as judged from the pattern of rRNAs visualized by gel electrophoresis through 1.5% agarose.

S1 nuclease mapping. The DNA probe was generated by digesting pJG500 (a pUC18 derivative containing the 498-bp *Nar*I-*Nar*I *sapA* gene fragment inserted in the *Acc*I site of the polylinker) with *Eco*RI (which cuts only in the pUC18 polylinker region) and *Bst*EII (which cuts in the *sapA* region), followed by treatment with calf intestinal alkaline phosphatase (11). The 245-bp *Eco*RI-*Bst*EII DNA fragment was purified by electroelution and ethanol precipitation from a 2% Tris-borate-EDTA agarose gel (11) and then labeled at its 5′ termini, using [γ-^{32}P]ATP (3,000 Ci/mmol) and polynucleotide kinase (12). Subsequent digestion with *Bam*HI generated two DNA fragments, an *Eco*RI-*Bam*HI fragment of 21 bp labeled at the 5′ position of the *Eco*RI terminus and containing only pUC18 polylinker DNA and a *Bam*HI-*Bst*EII fragment of 224 bp labeled at the 5′ position of the *Bst*EII terminus. The *Bam*HI-*Bst*EII fragment contained 13 bp from the pUC18 polylinker extending from *Bam*HI to *Acc*I and 211 bp of *S. coelicolor* DNA extending from the *Nar*I site upstream of the *sapA* coding sequence to the *Bst*EII site within the coding sequence.

S1 nuclease protection-hybridization experiments were carried out according to the method of Deng et al. (4), except that a hybridization temperature of 63°C was used, the hybridization was incubated only 4 h, and the S1 digestion was carried out with 100 U of S1 nuclease at 37°C for 30 min.

RESULTS

Identification of spore-associated polypeptides. To identify proteins associated with spore formation in *S. coelicolor*, we harvested mycelia at daily intervals during growth and development on solid sporulation medium. Protein was extracted with SDS and DTT and subjected to electrophoresis in an SDS-polyacrylamide gel. The time course experiment of Fig. 1, panel B, reveals two proteins of about 13 and 3 kDa whose time of appearance was approximately coincident with the time of appearance of aerial mycelia. Figure 1, panel A, shows that the 13- and 3-kDa species were also present in protein extracted from purified spores and were absent in vegetative mycelia grown in liquid medium. Interestingly, most of the spores apparently remained intact after SDS and DTT treatment, as judged by viability measurements and by microscopic examination. This finding, as well as the results of other experiments (not shown) in which the pattern of proteins present in sonically treated spores was examined, suggests that the 13- and 3-kDa proteins are located on the surface of the spore. We designated the 13- and 3-kDa proteins SapA and SapB, respectively.

Cloning the structural gene for SapA. To study the regulation of the spore-associated proteins, we attempted to clone their respective structural genes by means of a reverse genetic strategy based on a partial NH_2-terminal amino acid

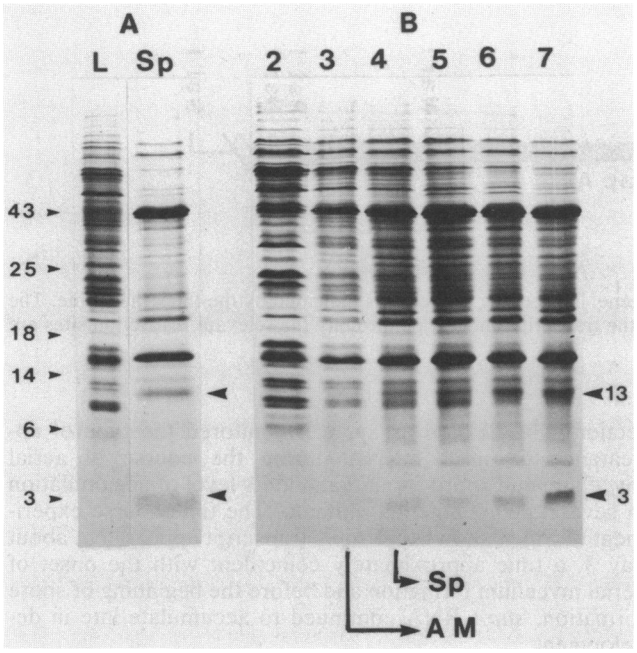

FIG. 1. SDS-polyacrylamide gel electrophoresis of proteins extracted at various stages of development. Protein was extracted from spores or mycelia with SDS and DTT and subjected to electrophoresis in an 18% polyacrylamide gel containing SDS. The gel was stained with Coomassie brilliant blue R250. The positions of molecular size markers are indicated in kilodaltons (at the left). The positions of SapA (13 kDa) and SapB (3 kDa) are indicated by arrows at the right. (A) Protein extracted from vegetative mycelia grown in liquid YEME for two days (L) and from purified spores (Sp); (B) protein extracted from cells grown on solid R2YE medium and harvested at the indicated daily intervals. Aerial mycelia (AM) appeared on day 3, and spores (Sp) appeared on day 4.

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                NarI                          Sau3A
5'    GGCGCCGAAGGGGCCTCCGGTGATCTTCTCGCGAACAGTCGTAAA

                                          **
CATCTGCCAACGACGTAACAACCCCCGAAGGTGCAAAGGTCTCAACTGGTGGCTTCAGCA

                                Sau3A
ACACAAGGGGAGGCCAGTCGATTCACGGATCGAGGTGCC ATG AAG CGT TCC ATG
                                         Met Lys Arg Ser Met

CAA GCC GTA GGG GCG ACG CTG ACC GCC GTC GGG GCC ATC GGG GCG
Gln Ala Val Gly Ala Thr Leu Thr Ala Val Gly Ala Ile Gly Ala

          BstEII
GGG CTG TTG GTG ACC GCC CCC GCC GCC GGC GCG GCG ACC GCG GGC
Gly Leu Leu Val Thr Ala Pro Ala Ala Gly Ala Ala Thr Ala Gly

GCG ACC GCG TCG TAC AAC GGG GTC TGC GGC TCC GGC TAC AAG GTG
Ala Thr Ala Ser Tyr Asn Gly Val Cys Gly Ser Gly Tyr Lys Val

                Sau3A
GTG AAC TCC ATG CCG ATC GGT TCC ACC GGC ACG GTC TAC CTC ACC
Val Asn Ser Met Pro Ile Gly Ser Thr Gly Thr Val Tyr Leu Thr

TAC AAC TCG GCC ACG GGC AAG AAC TGC ACC GTG ACC ATC CGC AAC
Tyr Asn Ser Ala Thr Gly Lys Asn Cys Thr Val Thr Ile Arg Asn

ACG ACG GCA CAC CGA CGT ACA TGG TGG CCT ACG TGC GCA ACA TCG
Thr Thr Ala His Arg Arg Thr Trp Trp Pro Thr Cys Ala Thr Ser

AAA GCG GCG CGG ACC AGT ACG ACG AGG GCG ACT ACC GGT CGT ACG
Lys Ala Ala Arg Thr Ser Thr Thr Arg Ala Thr Thr Gly Arg Thr

                                    NarI
CCC GGC CGG TCT ACT GTT CCG CCC GGC GCC   3'
Pro Gly Arg Ser Thr Val Pro Pro Gly Ala
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FIG. 2. Nucleotide sequence of the nontranscribed strand of the 5′ region of *sapA*. The position corresponding to the 5′ terminus of *sapA* RNA is indicated by the asterisks. The arrow shows the direction of transcription. The start codon and the putative ribosome-binding site are underlined and italicized. The NH_2 terminus of mature SapA protein as determined by Edman analysis is underlined.

sequence determination. Unfortunately, the SapB (3-kDa) polypeptide was refractory to sequence analysis, but automated Edman degradation yielded 19 amino acids of sequence for the SapA (13-kDa) protein (identified by underlining in Fig. 2). A synthetic 51-mer (see Materials and Methods) was designed and constructed on the basis of the NH_2-terminal sequence. We relied on the extreme G:C richness of *Streptomyces* DNA to choose the most likely base assignment in positions that were uncertain because of degeneracy of the code. Using the 51-mer as a probe, we identified, and cloned in *E. coli*, a 1.5-kilobase *Bam*HI-*Sal*I endonuclease restriction fragment that hybridized strongly with the synthetic oligonucleotide.

Southern hybridization analysis and DNA-subcloning experiments localized the region of hybridization of the 51-mer to a *Nar*I-*Nar*I DNA segment of 498 bp (Fig. 3), within which we located, by nucleotide sequence analysis, a stretch of DNA encoding the 19-amino-acid sequence at the NH_2 terminus of the 13-kDa polypeptide (Fig. 2). Interestingly, this sequence was 37 codons downstream of a putative initiation codon and putative ribosome-binding site. The predicted upstream amino acid sequence could be a signal peptide (possibly followed by a pro sequence, which is removed by secondary processing after removal of the signal peptide), as suggested by the presence of positively charged amino acids near the NH_2 terminus, followed by a long stretch of hydrophobic residues. Thus, the SapA protein could be a secreted protein that is extruded by and remains bound to the surface of the developing spores. The gene for SapA protein has been designated *sapA*.

Mapping the 5′ terminus of *sapA* mRNA. The mRNA for the *sapA* gene was found to originate from a unique 5′ terminus whose position was determined by means of nuclease-mapping experiments in which we used two kinds of hybridization probes. One probe was a radioactive RNA that was generated by runoff transcription of the 498-bp *Nar*I-*Nar*I fragment contained in a Riboprobe vector, using phage SP6 RNA polymerase. The other probe was a 224-bp *Bam*HI-*Bst*EII fragment of hybrid DNA that contained a short stretch of plasmid vector sequences at one end (a 13-bp *Bam*HI-*Acc*I segment of polylinker DNA) and a 211-bp stretch of *S. coelicolor* DNA extending from the *Nar*I site upstream of the *sapA* coding sequence to the *Bst*EII site within the coding sequence (Fig. 3). The DNA probe was uniquely labeled with [^{32}P]phosphate at the 5′ position of the *Bst*EII terminus.

The radioactive probes were hybridized with unlabeled RNA that had been purified from mycelia harvested at the time of spore formation. Next, after hybridization, the probes were treated with nuclease, and the resulting nuclease-resistant hybrids were denatured and subjected to electrophoresis in a polyacrylamide gel. Hybridization to the *Nar*I-*Nar*I Riboprobe and to the *Bst*EII end-labeled DNA probe yielded nuclease-resistant polynucleotides of approximately 400 (not shown) and 120 (Fig. 4) bases in length, respectively. Neither species was observed in control experiments in which the radioactive probes were hybridized with heterologous RNA from *Bacillus subtilis* (Fig. 4 and data not shown). As judged from the length of the nuclease-protected polynucleotides, we estimate that the DNA position corre-

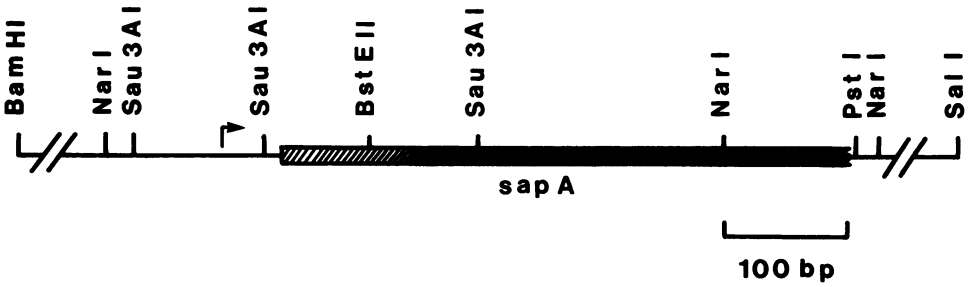

FIG. 3. Endonuclease restriction map of DNA in the vicinity of *sapA* gene. The coding sequence is indicated by the thick black line. The hatched region indicates the putative signal peptide. The arrow indicates the transcription start point. Only the relevant restriction sites are shown.

sponding to the 5′ terminus of *sapA* mRNA was located approximately 50 bp upstream of the initiation codon. An experiment to map the 5′ terminus to high resolution was carried out by subjecting the 120-base, nuclease-protected DNA to electrophoresis alongside base-specific cleavage products of the end-labeled DNA probe (Fig. 5). The position of the 5′ terminus is indicated by the asterisks in Fig. 2.

Temporal and developmental regulation of *sapA* transcription. Using the end-labeled DNA probe in a nuclease protection-hybridization assay, we monitored the time of appearance of *sapA* mRNA during the course of aerial mycelium and spore formation and its level of accumulation in several developmental mutants. The time course experiment of Fig. 4 shows that *sapA* transcript appeared at about day 3, a time approximately coincident with the onset of aerial mycelium formation and before the beginning of spore formation. *sapA* RNA continued to accumulate late in development.

To investigate further the correlation of *sapA* expression with morphological differentiation, we examined the level of

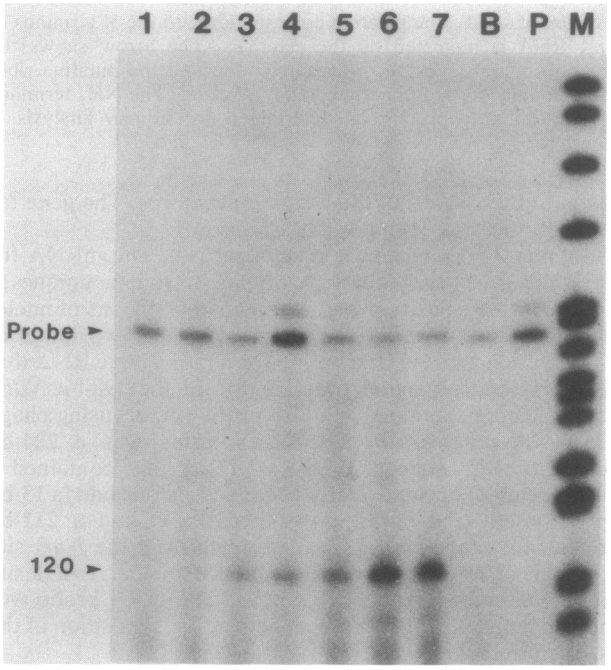

FIG. 4. Use of the nuclease protection-hybridization assay to monitor the time of appearance of *sapA* RNA during development on solid medium. Nuclease protection-hybridization assays were carried out, using the end-labeled, 224-bp *Bam*HI-*Bst*EII DNA (10 ng), described in Materials and Methods, as the probe and unlabeled RNAs (40 μg) that had been purified from cells grown over nitrocellulose filters on solid R2YE medium and harvested at the indicated daily intervals (tracks 1 through 7). As a negative control, track B shows the result of hybridizing the probe to *B. subtilis* RNA. The radioactive probe is shown in track P. The position of molecular weight markers generated by ^{32}P-labeled, *Hpa*II-digested pBR322 DNA (22) are displayed in track M. The "shadow" over the probe in tracks 1 through 7 and B and P corresponds to incomplete digestion of the *Eco*RI-*Bst*EII DNA used to generate the *Bam*HI-*Bst*EII end-labeled probe (see Materials and Methods).

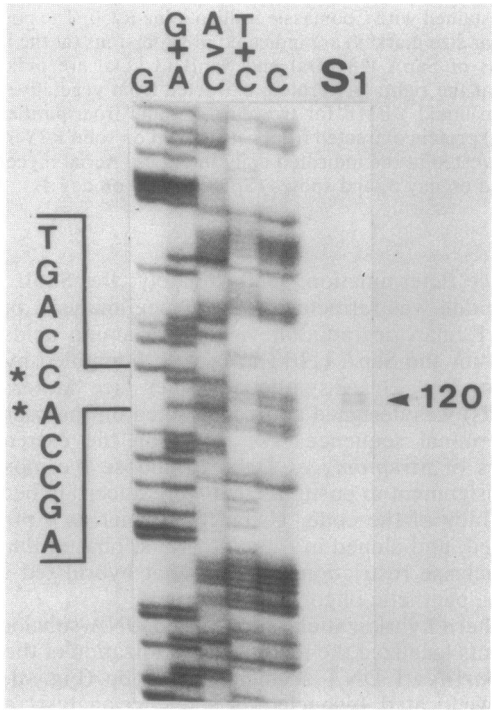

FIG. 5. S1 nuclease mapping of the 5′ terminus of *sapA* RNA. Radioactive probe DNA that had been uniquely 5′ end labeled at the *Bst*EII terminus within the *sapA* coding sequence (prepared as described in Materials and Methods) was denatured and hybridized with unlabeled RNA purified from 4-day-old cultures of cells undergoing spore formation. The resulting RNA-DNA hybrids were treated with S1 nuclease, denatured, and subjected to high-resolution gel electrophoresis alongside base-specific cleavage products (12) of the end-labeled probe. The asterisks indicate the positions of the 5′ termini as estimated by correcting by about 1.5 bases for the fact that the reactions of Maxam and Gilbert (12) destroy the terminal base and leave a 3′ phosphate.

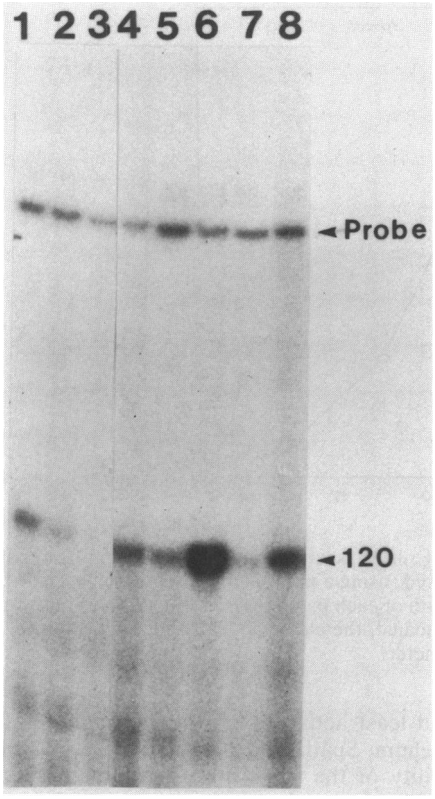

FIG. 6. Use of the nuclease protection-hybridization assay to monitor the level of *sapA* RNA in developmental mutants. RNA was prepared from cells harvested after 7 days on solid medium from the following mutant strains: J1700 (*bldA39*; track 1), J669 (*bldB43*; track 2), J660 (*bldC18*; track 3), C70 (*whiB70*; track 4), C107 (*whiE107*; track 5), J1820 (*whiG71*; track 6), J95 (*whiH119*; track 7), and A3(2) (wild type; track 8). The nuclease protection-hybridization assays were carried out as described in Materials and Methods and the legends to Fig. 4 and 5.

sapA transcript in a collection of nonisogenic developmental mutants. The collection included mutants blocked in aerial mycelium formation (*bld* mutants) and mutants that produce aerial mycelia but are unable to form spores (*whi* mutants). Although *sapA* RNA was clearly present in almost all of the mutants, the level of transcript was significantly reduced in *bldC* (Fig. 6, track 3), *bldD* (not shown), and *whiH* (Fig. 6, track 7) strains. (The over-accumulation of *sapA* RNA observed in the *whiG* strain [track 6, Fig. 6] was not reproducible.)

Use of the *lux* reporter genes to visualize *sapA* gene expression temporally and spatially. In other work, we (A. Schauer, M. Ranes, R. Santamaria, J. Guijarro, E. Lawlor, C. Mendez, K. Chater, and R. Losick, manuscript in preparation) have developed the luciferase-encoding *luxA* and *luxB* operon of *Vibrio harveyi* as a promoter probe in *S. coelicolor*. The substrate for bacterial luciferase is a volatile aldehyde that is noninhibitory for growth and development and can be supplied exogenously as a vapor. The use of the *lux* operon as a promoter probe is highly advantageous for studies of developmental gene regulation in streptomycetes, because colonies of *lux* operon fusion-containing cells can be monitored for: (i) the time during development when luciferase synthesis is induced and (ii) the location of light-emitting cells in colonies undergoing morphological differentiation.

Elsewhere, we (Schauer et al., manuscript in preparation) describe the construction of a *Streptomyces* plasmid vector called pRS1105 that contains a promoterless *luxAB* operon preceded by a strong transcription terminator. The terminator effectively prevents transcriptional readthrough from the vector into the *lux* genes, and little expression of the *lux* operon is observed in *S. coelicolor* cells containing pRS1105. To place the *luxAB* operon under the control of the *sapA* promoter, we constructed a *sapA-luxAB* fusion by cloning a 111-bp *Sau*3AI-*Sau*3AI fragment containing the 5′ end of the *sapA* transcription unit (Fig. 3) into a unique *Bam*HI site located between the terminator and the *luxAB* genes in pRS1105. The resulting *sapA-luxAB*-containing plasmid is designated pS960. Colonies of pS960-bearing cells were grown on solid sporulation medium and monitored for the induction of luciferase synthesis at daily intervals during the course of development by use of a photometer. The time course experiment of Fig. 7 shows that luciferase synthesis commenced on or after the time of appearance of aerial mycelium formation, a finding in agreement with the time of appearance of the *sapA* transcript as determined by use of the nuclease protection-hybridization assay.

As a further test of the association of *sapA* expression with aerial mycelium formation, we attempted a topographical correlation of *sapA*-directed luciferase synthesis with regions of aerial mycelium formation in colonies of pS960-bearing cells. Streptomycete colonies characteristically undergo development unevenly, with certain well-demarcated zones undergoing aerial mycelium formation more rapidly than other regions of the colony. The photograph in Fig. 8A records light emission from colonies of pS960-bearing cells. It can be seen that *sapA*-directed luciferase synthesis was concentrated in well-defined zones around the outside of the colonies, which were coincident with regions of aerial mycelium as judged by inspection with overhead light (the whitish grey regions in Fig. 8B).

DISCUSSION

We have cloned a gene called *sapA* encoding a 13-kDa protein (SapA) associated with the spores of *S. coelicolor*. SapA appears to be located on the spore surface, because it could be solubilized under conditions which did not cause measurable spore lysis. Consistent with the apparent surface localization of SapA, the nucleotide sequence of its structural gene suggests that the primary product of *sapA* is a pre- or preproprotein in which the 13-kDa polypeptide is preceded by a signal sequence or a combination of signal and pro sequence of 37 amino acids. An example of a secreted *Streptomyces* protein with a similarly long prepro sequence (42 residues) that, like SapA, has positively charged amino acids near its predicted NH_2 terminus is the endo-β-*N*-glycosaminidase H enzyme of *Streptomyces plicatus* (20).

Using the cloned *sapA* gene as a hybridization probe, we have shown that *sapA* mRNA originates from a unique site located 50 bp upstream from the preprotein-coding sequence. Experiments in which we used *luxAB* as a promoter probe demonstrated that the functional boundaries of the *sapA* promoter were within a 111-bp *Sau*3AI-*Sau*3AI DNA segment that extended 72 bp upstream (and 39 bp downstream) from the site from which *sapA* transcripts originate. Transcription from the *sapA* promoter appeared to be subject to temporal and developmental regulation, as judged from experiments in which the appearance of transcripts originating from the *sapA* promoter was found to coincide with the time of appearance of aerial mycelia and from experiments in which the abundance of these transcripts was found to be substantially reduced in certain developmental mutants.

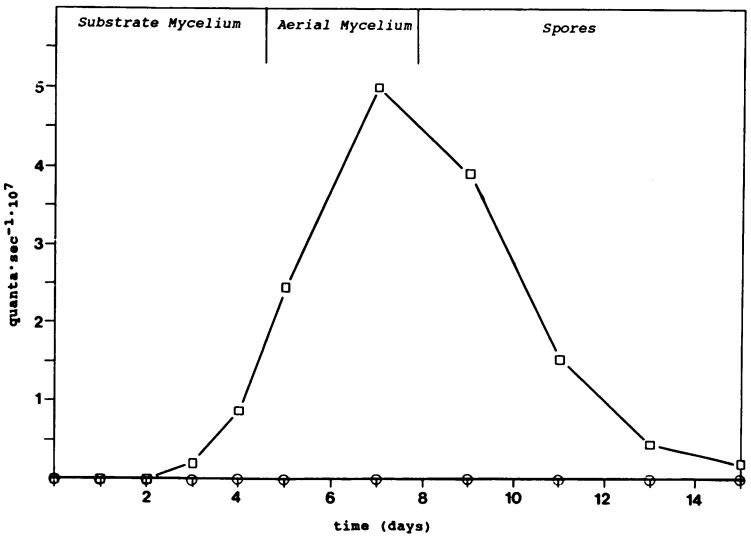

FIG. 7. Use of a fusion to the *luxAB* reporter genes to monitor the time course of *sapA* transcription. Light emission from patches of *S. coelicolor* cells bearing plasmid pS950 containing the *sapA-luxAB* fusion was measured, using a photometer, as will be described elsewhere (Schauer et al., manuscript in preparation). At each time point, a homogeneous portion of each patch (of uniform surface area) was removed from an agar plate and transferred to a glass scintillation vial containing 2 μl of *n*-decanal, the aldehyde substrate for luciferase. The patch was oriented such that its upper surface was facing the input window of the photometer.

As further evidence that transcription of *sapA* was associated with aerial mycelium formation, experiments in which we monitored gene expression topographically by use of the *sapA-luxAB* operon fusion demonstrated that transcription of *sapA* was restricted spatially to colony regions that had undergone aerial mycelium formation. Thus, transcription of *sapA* was most active in regions where aerial mycelia were present and least active in regions consisting only of substrate mycelium. Spatially restricted expression was not due to an inability of the substrate mycelium to support transcription of *lux* genes, because in other work (A. Schauer, unpublished experiments) we have demonstrated light emission from the substrate mycelium by using certain other *lux* fusions.

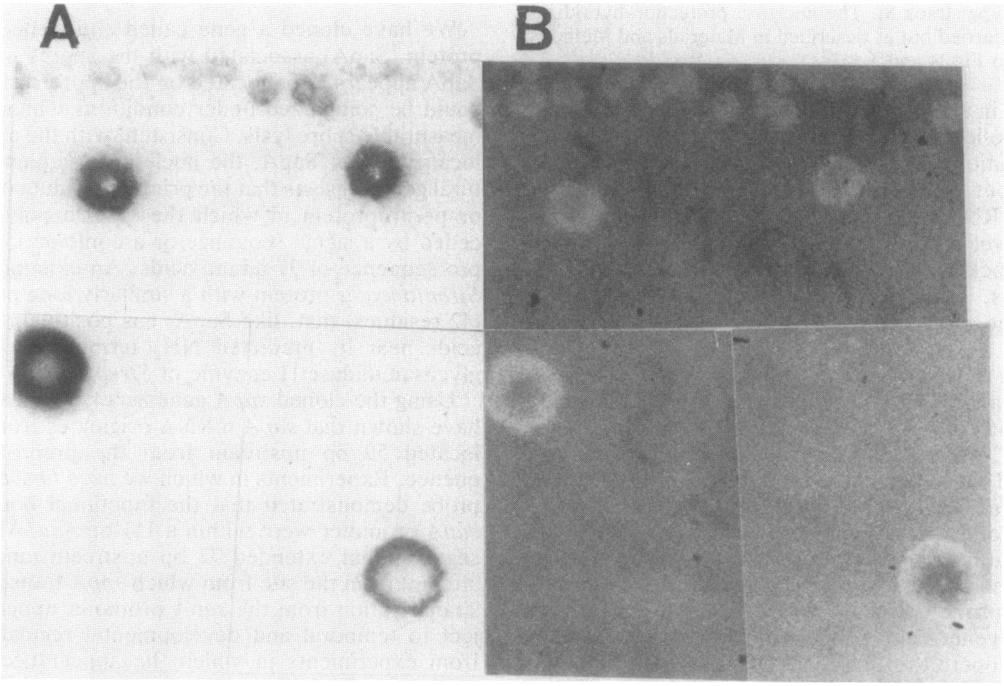

FIG. 8. Use of a fusion of the *luxAB* reporter genes to localize *sapA* transcription spatially. (A) Photograph (negative) of light emission from colonies of *S. coelicolor* cells bearing plasmid pS950 after exposure to *n*-decanal. The image was obtained by use of an image intensifier, as will be described elsewhere (Schauer et al., manuscript in preparation). (B) Floodlight image of the same colonies. At the time the photographs were taken, the colonies were whitish grey (indicative of aerial mycelium) at their edges and pale yellow (substrate mycelium) at their centers.

In toto, these experiments suggest that under conditions of morphological differentiation on solid medium, the expression of *sapA* is temporally, genetically, and spatially linked to the formation of aerial mycelia. Under other circumstances, however, *sapA* expression can be uncoupled from morphological differentiation. In some of the *bld* mutants studied in which, by definition, aerial mycelium formation does not take place, transcription of *sapA* was found to occur at a level comparable to that observed in wild-type cells. Also, in other work (not shown) we have found that *sapA* can be strongly induced in liquid medium after cells enter stationary phase. In *S. coelicolor*, morphological development does not occur in liquid medium. Thus, although expression of *sapA* is associated with aerial mycelium formation, morphological differentiation is not a prerequisite for activation of the *sapA* gene, at least under certain conditions.

The *sapA* gene should be a useful tool for future genetic and biochemical studies of gene expression during morphological differentiation in *S. coelicolor*. The use of the *luxAB* transcription fusion to visualize *sapA* gene expression should facilitate a genetic approach to the problem of temporal and spatial control of gene expression. Also, the use of a cloned copy of the *sapA* gene as a template for in vitro transcription experiments may, in extension of earlier work (24), provide a biochemical approach to the identification of factors involved in its transcription and proper regulation.

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LITERATURE CITED

1. **Binnie, C., M. Lampe, and R. Losick.** 1986. Gene encoding the σ^{37} species of RNA polymerase σ factor from *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **83**:5943–5947.
2. **Chater, K. F.** 1972. A morphological and genetic mapping study of white colony mutants of *Streptomyces coelicolor*. J. Gen. Microbiol. **72**:9–28.
3. **Chater, K. F.** 1984. Morphological and physiological differentiation in *Streptomyces*, p. 89–116. *In* R. Losick and L. Shapiro (ed.), Microbial development. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Deng, Z., T. Kieser, and D. A. Hopwood.** 1986. Expression of a *Streptomyces* plasmid promoter in *Escherichia coli*. Gene **43**: 295–300.
5. **Donovan, W., L. Zheng, K. Sandman, and R. Losick.** 1987. Genes encoding spore coat polypeptides from *Bacillus subtilis*. J. Mol. Biol. **196**:1–10.
6. **Goldman, R. C., and D. J. Tipper.** 1978. *Bacillus subtilis* spore coats: complexity and purification of a unique polypeptide component. J. Bacteriol. **135**:1091–1106.
7. **Hopwood, D. A.** 1987. Genetic analysis and genome structure in *Streptomyces coelicolor*. Bacteriol. Rev. **31**:373–403.
8. **Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, J. M. Ward, and H. Schrempf.** 1985. Genetic manipulation of *Streptomyces*: a laboratory manual. John Innes Institute, Norwich, England.
9. **Hunkapeller, M. W., E. Lujan, F. Ostrander, and L. E. Hood.** 1983. Isolation of microgram quantities of proteins from polyacrylamide gels for amino acid sequence analysis. Methods Enzymol. **91**:227–236.
10. **Igo, M., and R. Losick.** 1986. Regulation of a promoter that is utilized by minor forms of RNA polymerase holoenzyme in *Bacillus subtilis*. J. Mol. Biol. **191**:615–624.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
13. **Mendez, C., and K. F. Chater.** 1987. Cloning of *whiG*, a gene critical for sporulation of *Streptomyces coelicolor* A3(2). J. Bacteriol. **169**:5715–5720.
14. **Merrick, M. J.** 1976. A morphological and genetic mapping study of bald colony mutants of *Streptomyces coelicolor*. J. Gen. Microbiol. **96**:244–315.
15. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Mills, D. R., and F. R. Kranier.** 1979. Structure-independent nucleotide analysis. Proc. Natl. Acad. Sci. USA **76**:2232–2235.
17. **Mizusawa, S., S. Nishimura, and F. Seelu.** 1986. Improvement of the dideoxy chain termination method of DNA sequencing by use of deoxy-7-deazaguanosine triphosphate in place of dGTP. Nucleic Acids Res. **14**:1319–1324.
18. **Penn, M. D., G. Thireos, and H. Greer.** 1984. Temporal analysis of general control of amino acid biosynthesis in *Saccharomyces cerevisiae*: role of positive regulatory genes in initiation and maintenance of mRNA derepression. Mol. Cell. Biol. **4**:520–528.
19. **Piret, J. M., and K. F. Chater.** 1985. Phage-mediated cloning of *bldA*, a region involved in *Streptomyces coelicolor* morphological development, and its analysis by genetic complementation. J. Bacteriol. **163**:965–972.
20. **Robbins, P. W., R. Trimble, D. F. Wirth, C. Herin, F. Maley, G. F. Maley, R. Das, B. W. Gibson, N. Royal, and K. Bieman.** 1984. Primary structure of the *Streptomyces* enzyme endo-β-*N*-acetylglucosaminidase H. J. Biol. Chem. **259**:7577–7583.
21. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
22. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
23. **Thomas, J. O., and R. D. Kornberg.** 1978. The study of histone-histone associations by chemical cross-linking. Methods Cell Biol. **18**:429–440.
24. **Westpheling, J., M. Ranes, and R. Losick.** 1985. RNA polymerase heterogeneity in *Streptomyces coelicolor*. Nature (London) **313**:22–27.
25. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–109.