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Transcriptional Regulation of *Streptomyces coelicolor* Pathway-Specific Antibiotic Regulators by the *absA* and *absB* Loci

DAVID J. ACETI† AND WENDY C. CHAMPNESS*

Department of Microbiology, Michigan State University, East Lansing, Michigan 48824-1101

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The four antibiotics produced by *Streptomyces coelicolor* are all affected by mutations in the *absA* and *absB* loci. The *absA* locus encodes a putative two-component signal transduction system, and the *absB* locus encodes a homolog of *Escherichia coli* RNase III. We assessed whether these loci control synthesis of the antibiotics actinorhodin and undecylprodigiosin by regulating transcript abundance from the biosynthetic and regulatory genes specific for each antibiotic. Strains that were Abs$^-$ (for antibiotic synthesis deficient) due to mutations in *absA* or *absB* were examined. In the Abs$^-$ *absA* mutant strain, transcripts for the actinorhodin biosynthetic genes *act*VI-ORF1 and *act*I, and for the pathway-specific regulatory gene *act*II-ORF4, were substantially lower in abundance than in the parent strain. The level of the transcript for the undecylprodigiosin pathway-specific regulatory gene *redD* was similarly reduced in this mutant. Additionally, a strain that exhibits precocious hyperproduction of antibiotics (Pha phenotype) due to disruption of the *absA* locus contained elevated levels of the *act*VI-ORF1, *act*II-ORF4, and *redD* transcripts. In the *absB* mutant strain, *act*VI-ORF1, *act*I, *act*II-ORF4, and *redD* transcript levels were also substantially lower than in the parent strain. These results establish that the *abs* genes affect production of antibiotics through regulation of expression of the antibiotic-specific regulatory genes in *S. coelicolor*.

Bacteria of the genus *Streptomyces* produce most of the antibiotics currently used in medicine. Bacterial cultures synthesize these compounds as secondary metabolites such that production follows the rapid growth phase of liquid-grown cultures or is coupled to sporulation of plate-grown cultures. Despite the many years of research on antibiotics driven by their commercial importance, the regulatory mechanisms responsible for antibiotics' growth phase regulation are poorly understood.

Streptomyces coelicolor, the streptomycete best understood genetically, produces four biochemically and genetically distinct antibiotics: actinorhodin (Act [48]), undecylprodigiosin (Red [18, 48]), methylenomycin (Mmy [38, 56]), and calcium-dependent antibiotic (CDA [28]). The gene clusters responsible for Act (39), Red (18, 40), CDA (15a), and Mmy (13) synthesis have been cloned. The *act* cluster is the best characterized of these and consists of at least six transcripts and 20 open reading frames (ORFs). Transcription of the *act* biosynthetic genes is regulated by a cluster-linked regulatory gene, *act*II-ORF4 (19). The *red* cluster also contains a regulator, *redD* (42, 52), which is in turn regulated by another cluster-linked regulator, *redZ* (54). The sequences of ActII-ORF4 and RedD have considerable similarity at the amino acid level and appear to contain an OmpR-like DNA-binding fold (55). Both *act*II-ORF4 and *redD* are growth phase regulated; their transcripts accumulate at approximately the onset of stationary phase (25, 52). Shortly thereafter, accumulation of biosynthetic gene transcripts is seen (25, 52). ActII-ORF4 and RedD have been called "pathway-specific regulators," and the evidence accumulated thus far indicates that they are positive regulators for the respective biosynthetic genes. For example, strains carrying mutant genes fail to accumulate biosynthetic gene transcripts and fail to cosynthesize Act (reviewed in reference 14) or Red (18, 49), respectively, with other *act* or *red* mutant classes. Moreover, overexpression of *act*II-ORF4 or *redD* early in a culture's growth leads to early biosynthetic gene transcription and antibiotic production (25, 52). Thus, growth-phase-regulated transcription of antibiotic-specific regulators is one aspect of antibiotics' temporal regulation.

A genetic analysis of *S. coelicolor* antibiotic regulation that sought to identify genes potentially involved in global temporal regulation of antibiotics defined the *absA* (2) and *absB* (1) loci. Both loci were defined by mutations that caused a phenotype of global loss of antibiotic synthesis (Abs$^-$ [for antibiotic synthesis deficient]). Hence these loci mutated to phenotypes that suggested that they encode global regulators of antibiotic synthesis.

The sequence of *absA* is predicted to encode a two-component signal transduction system composed of AbsA1, a putative sensor-transmitter, and AbsA2, a putative DNA-binding response regulator (5). The original collection of Abs$^-$ mutant strains (2), which was obtained from UV mutagenesis, carried mutations of the *absA1* gene (5). In contrast, disruptions of the *absA1A2* genes result in a phenotype of early-onset enhanced antibiotic production (Pha [for precocious hyperproduction of antibiotics]) (5). Hence, we hypothesize that the *absA* locus plays a negative regulatory role in antibiotic production and that the Abs$^-$ *absA* strains are mutationally locked into a negatively acting mode of regulation (5).

The sequence of *absB* is predicted to encode a homolog of *Escherichia coli* RNase III, a double-stranded-RNA-specific endonuclease (45). RNase III processes a number of mRNAs of *E. coli* and coliphage and thus regulates expression of numerous genes (17, 43). *E. coli* RNase III is also involved in rRNA processing, but this is a nonessential activity (17, 43).

A number of additional loci that are relevant to the regulation of *S. coelicolor*'s antibiotics have been identified. The serine-threonine-tyrosine phosphotransfer system-encoding *afsRKS* locus stimulates Act and Red synthesis when in high copy, while disruptions of genes encoded by the locus lead to

* Corresponding author. Mailing address: Department of Microbiology, Michigan State University, East Lansing, MI 48824-1101. Phone: (517) 353-9770. Fax: (517) 353-8957. E-mail: champnes@pilot.msu.edu.

† Present address: Department of Biochemistry, University of Wisconsin—Madison, Madison, WI 53706-1569.

medium-dependent reductions of Act, Red, and CDA (23, 27, 31–34, 53). The recently described *cutRS* putative two-component system appears to be a negative regulator of Act, since gene disruption causes overproduction of that antibiotic (11). The *afsQ1Q2* genes, which encode another putative two-component system, stimulate Act and Red (36). Other, less-well-characterized loci influence one, two, or three of the antibiotics: *afsB* (affecting Act and Red [26]), *abaA* (Act, Red, and CDA [20]), and the *abaB* (Act and Red [51]) and "Romero" sequences (Act [47]). Synthesis of the compound (p)ppGpp by the *relA* gene product has been tied to the induction of antibiotic synthesis (reviewed in reference 4). RNA polymerase sigma factors are also potentially important to the regulation of antibiotic synthesis, but the sigma factor(s) recognizing antibiotic gene promoters has not been definitively specified. The sigma factor σ^{hrdD} transcribes *act*II-ORF4 and *redD* in vitro but is dispensible in vivo. The essential vegetative sigma factor σ^{hrdB} (7, 24) is a strong candidate for in vivo transcription. Finally, streptomycete antibiotic production is temporally coupled to sporulation, and numerous genetic loci named *bld* can mutate to a phenotype characterized by loss of both antibiotic production and sporulation (reviewed in references 10 and 30).

The mechanisms by which the above-mentioned genes influence antibiotic production have not been well defined, but some types of mutants blocked in Act and Red production have been evaluated for *act*II-ORF4 and *redD* transcription. *bldA* encodes the only tRNA of *S. coelicolor* that can efficiently translate the rare leucine codon UUA, which is found in the *act*II-ORF4 gene and the *redZ*-encoded regulator of *redD*. Hence, *bldA* mutants are Red$^-$ because of diminished *redD* transcription (54) but are Act$^-$ because of defective *act*II-ORF4 translation (19).

Some strains carrying mutations in the *afsRK* locus, including a deletion mutant of *afsR* (23), show a reduction in transcription of biosynthetic transcripts for Act but no effects on the transcripts for *act*II-ORF4 or *redD*. Finally, some strains with mutations in *relA*, which encodes (p)ppGpp synthetase, are affected in actinorhodin and undecylprodigiosin production with accompanying defects in *act*II-ORF4 and *redD* transcription (8, 41).

Here, we report a characterization of the regulation of *act* and *red* transcripts by the *absA* and *absB* loci. Using S1 nuclease protection assays, we show that both *absA* and *absB* are regulators of antibiotic biosynthetic gene expression and, moreover, are global regulators of expression of the antibiotic pathway-specific activators.

MATERIALS AND METHODS

Bacterial strains. The following *S. coelicolor* A3(2) strains were used: J1501 (15) and its derivatives C542 (*absA542* [2]), C120 (*absB120* [1]), J1501/KC900 (*act*I::KC900 [5, 6]), C542/KC900, and C120/KC900.

XylE enzyme assays. Growth conditions and assay techniques for the KC900 lysogens were as described previously (35) except that XylE enzyme activity was assayed on R5-thiostrepton plates. The KC900 phage (6) contains a fragment internal to the *act*I coding region and creates lysogens through homologous recombination with *act*I. Thus, it creates a single-copy transcriptional fusion of the *act*I promoter to the reporter gene *xylE* (6). Color development was visually evaluated 1 h after spraying with catechol.

Growth conditions and RNA isolation. *S. coelicolor* strains were grown for RNA isolation on 8.5-cm-diameter cellophane disks (Cannings Packaging Ltd., Bristol, England) placed on plate media. Disks were washed by autoclaving twice for 15 min in 1 to 2 liters of distilled water. Two media were used for RNA isolation: a mannitol minimal medium (9) and a peptone-glucose (PGA) medium (0.5% peptone, 1.0% glucose, 2.2% agar [pH 7.1] [modified from reference 16]). The experiment shown in Fig. 1 used mannitol minimal medium, but other experiments used PGA medium because it allowed reproducible production of both Act and Red by cultures grown on cellophane disks. A variety of other medium formulations were surveyed, but they were not used, because only one antibiotic was produced. In some cases, certain media supported antibiotic production only in the absence of cellophane disks. Approximately 10^5 spores were spread onto each disk, followed by incubation at 30°C. RNA was harvested at time points postinoculation chosen to span the initiation of antibiotic synthesis; these are stated for each experiment. In a 4°C cold room, growth from each of 8 to 10 disks was scraped into a tube containing 5 ml of chilled modified Kirby mixture (29) and 14 g of 4-mm-diameter glass beads. Tube contents were alternately mixed vigorously for 30 s by vortex mixer and incubated on ice for 30 s, for four cycles. Samples were then processed as previously described for Northern blot RNA preparation (29). RNA quality was tested by gel electrophoresis and ethidium bromide staining; little or no degradation of rRNA was evident.

S1 nuclease protection assays. For each assay, 50 μg of RNA was dried down with the appropriate ^{32}P-labeled DNA probes (30,000 or 100,000 cpm of restriction digest-generated or PCR-generated probes, respectively). Pellets were suspended in 20 μl of 80% formamide buffer (29) by pipetting and vortex mixing. Tubes were placed in a water bath that was kept at 85°C for 10 min then allowed to cool to 57°C overnight. Samples were processed as described previously (29) except as follows: S1 nuclease buffer was that described by Sambrook et al. (50); isopropanol precipitations were incubated on ice for 30 min; and final pellets were dissolved in standard sequencing gel-loading buffer (50), boiled for 2 min, and electrophoresed on denaturing 6% polyacrylamide sequencing gels (50). Size markers were purchased from New England Biolabs, Beverly, Mass. In concert with the experiments shown, control experiments in which samples of total RNA were hybridized with twice the probe concentration used in other lanes were performed; the control signals were not significantly different from corresponding experimental signals, confirming that probes were present in excess (data not shown). Control experiments in which 50 μg of yeast tRNA replaced experimental total RNA samples were performed with each set of S1 nuclease protection assays; no signals resulted (data not shown). Radioactivity on gels was quantitated with an AMBIS Radioanalytic Imaging System and AMBIS Quantprobe, version 3.0, software (AMBIS, Inc., San Diego, Calif.). Bands on autoradiograph films were quantitated by densitometry with a Molecular Dynamics (Sunnyvale, Calif.) computing densitometer and ImageQuant, version 3.0, software. A *glk* probe was generated by PCR amplification with the labeled 3′ primer 5′-GATGCCCACTGCGACGATCT-3′ and the unlabeled 5′ primer 5′-CCAGATCTGCAGCCAAGCTT-3′ to produce a 309-bp fragment that included the *glk* promoter region (3). The PCR template was pIJ2423, which carries the 1.2 kb *Sma*I-(*Bcl*I)-*Hin*dIII *glk* fragment from pIJ2420 (3) blunt-ended and cloned into the *Sma*I site of pIJ2925 (37). Two *glk* signals were seen; those labeled *glk* (3) represent transcript from the *glk* promoter, and those labeled *glk* (2 + 3) represent readthrough from an upstream promoter. In most time courses, the average *glk* band intensities were comparable for the J1501 and *abs* strains. In some time courses, e.g., that shown in Fig. 2, the *glk* band intensities dropped at very late times in all strains. The transcript abundances in *abs* mutant strains relative to the J1501 parent were determined by comparing the band intensities of the antibiotic gene transcripts in question to the *glk* band intensities. An *act*II-ORF4 probe was made from a 466-bp *Xho*I/*Ase*I DNA fragment that included the promoter region of *act*II-ORF4 (25). The fragment was uniquely labeled at the 5′ end of the *Xho*I site with [^{32}P]ATP by using T4 polynucleotide kinase. The *act*VI-ORF1 probe was generated by PCR amplification of a sequence that included the promoter region of that gene (22) by using the end-labeled 3′ oligonucleotide primer 5′-ACGTCCGGCTCGTACTCGATG-3′ and the unlabeled 5′ primer 5′-CTTGCGGTGGAAGTCCTCCAG-3′. The PCR template was a 4.5-kb *Bam*HI *act* fragment (sites 1 to 3 in reference 22). A *redD* probe was made with the 3′ oligonucleotide primer 5′-ACAGTTCGTCCACCAGGTCCGCGA-3′ (end labeled before use) in the PCR with the unlabeled 5′ primer 5′-TGCTTCGTTTGCGTCGTTCAGTTC-3′ to generate a 497-bp fragment that included the *redD* promoter region (42, 52). The PCR template was the 2.1-kb *redD* fragment in pCLL38 (42) cloned into pUC18. The PCR mix contained 1× PCR buffer lacking $MgCl_2$ (Perkin-Elmer), 2 mM $MgCl_2$, 1% glycerol, 0.2 to 0.4 mM (each) deoxynucleoside triphosphate, 20 pmol of each primer, 100 ng of template DNA, and 2.5 U of *Taq* polymerase (Perkin-Elmer). Formamide at 2% (vol/vol) was included for *redD* amplification. Samples were subjected to 29 (*act*VI-ORF1 and *glk*) or 36 (*redD*) cycles of 3 min at 95°C, 2 min at 65°C, and 1 min at 72°C, and a final extension of 10 min at 72°C. The 497-nucleotide (nt) *redD* product was further purified from contaminating PCR products by agarose gel electrophoresis and isolation.

RESULTS

Regulation of the Act-specific regulator *act*II-ORF4 by the *absA* and *absB* loci. Because the Abs$^-$ mutant phenotypes suggested that *absA* and *absB* were regulatory loci, transcription of antibiotic genes was assessed in *absA* and *absB* mutant strains and in the parental strain J1501 (*abs*$^+$). The expression profile of *act* transcripts has been especially well characterized (25), and current evidence indicates that *act*II-ORF4 is the most directly acting regulator of the Act biosynthetic transcripts (19, 25, 44). The *act*II-ORF4 transcript is growth phase regulated in liquid-grown cultures of *S. coelicolor*, its level increasing greatly in the transition and stationary phases (25).

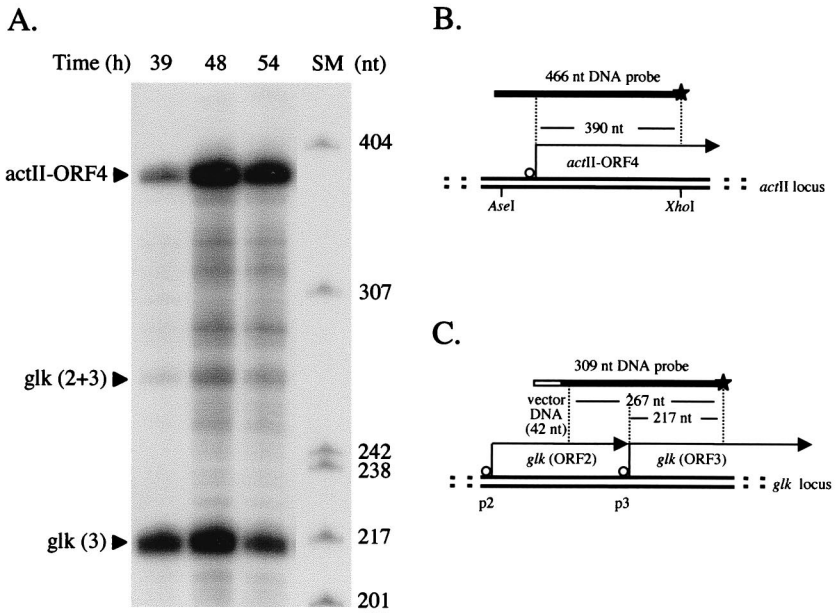

FIG. 1. Growth-phase-dependent expression of *act*II-ORF4 mRNA in plate-grown J1501. (A) RNA isolated from mannitol minimal medium plates at the times indicated was used in S1 nuclease protection analyses. Size markers (SM), ^{32}P-labeled *Msp*I-digested pBR322. (B) The probe for the *act*II-ORF4 gene was end labeled at the *Xho*I site (indicated by a star; see Materials and Methods) and should yield a protected fragment of 390 nt (19, 25). (C) The uniquely end-labeled probe for *glk* was generated by PCR amplification (see Materials and Methods) and should yield two protected fragments of 267 and 217 nt that correspond to transcripts initiated at promoters p2 and p3, respectively (3).

Figure 1A shows that plate-grown cultures of strain J1501 (Abs$^+$) also exhibit temporal regulation of *act*II-ORF4 transcription, as assessed by S1 nuclease protection analysis of isolated RNA. In this experiment, Act was made visibly at 48 hours, approximately coordinated with sporulation.

The nuclease protection experiments included an assessment of the *S. coelicolor* glucose kinase transcript level. The glucose kinase gene (*glk* [ORF3] in Fig. 1C) is expressed throughout the growth of *S. coelicolor* from two promoters (3), referred to as p2 and p3 in Fig. 1C. The abundance of transcript from p2 is much lower than that from p3. Each S1 assay included an assay for *glk* mRNA, providing an internal control for in vivo RNA levels and the S1 procedure. The interpretations of relative transcript levels in the experiments discussed below reflect adjustments based on this control (see Materials and Methods).

Figure 2 shows a comparison of *act*II-ORF4 transcript levels in J1501, C542 (*absA*), and C120 (*absB*). In this time course, Act was produced by J1501 at about 42 h. For this experiment, and those following, PGA plate medium was used because J1501 reproducibly produced both Act and Red when grown with PGA on cellophane disks.

In comparison to J1501, *act*II-ORF4 transcript abundance in C542 was substantially reduced over the course of the experiment. The maximum level in C542, observed at 54 h in this experiment, was approximately 25% of the level in J1501. In four similar experiments involving two independent time courses of RNA isolation, the *act*II-ORF4 transcript levels were decreased approximately three- to sixfold in C542 (data not shown).

Figure 2 also shows that, in comparison to J1501, *act*II-ORF4 transcript abundance was reduced about 12-fold in the *absB* strain C120. In four similar experiments involving two independent time courses of RNA isolation, the *act*II-ORF4 transcript levels were decreased approximately two- to sixfold in C120 (data not shown).

Note that the abundance of the signal at the position of the *act*II-ORF4 probe generally follows the same pattern as that indicating probe protection due to transcription from the *act*II-ORF4 promoter. This most likely reflects coregulation of the upstream transcript (*act*II-ORF3 [19]) with *act*II-ORF4.

Regulation of Act biosynthetic genes by the *absA* and *absB* loci. To assess the extent to which the reduced *act*II-ORF4 expression in strains C542 and C120 affected expression of *act* biosynthetic genes, two representative *act* transcripts were studied: *act*VI-ORF1, proposed to encode a dehydrogenase

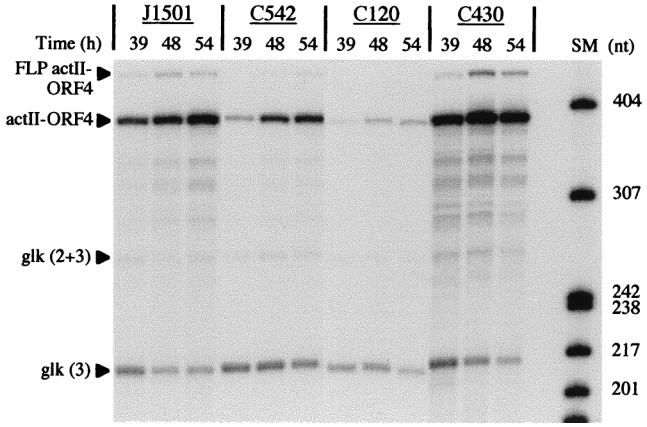

FIG. 2. Expression of *act*II-ORF4 mRNA in J1501 (*abs*$^+$), C542 (*absA*), C120 (*absB*), and C430 (*absA* disruption). RNA was isolated from PGA plate-grown cultures at the times indicated and used for S1 nuclease protection analyses. The size markers (SM) used were as described in the legend to Fig. 1. The probes used for *act*II-ORF4 and *glk* were the same as for Fig. 1. "FLP *act*II-ORF4" indicates the position of full-length probe; transcriptional readthrough from the upstream promoter for *act*II-ORF3 likely contributes to the signal as well (19, 25). This signal intensity varied from experiment to experiment but, when present, followed the same kinetics as the labeled *act*II-ORF4.

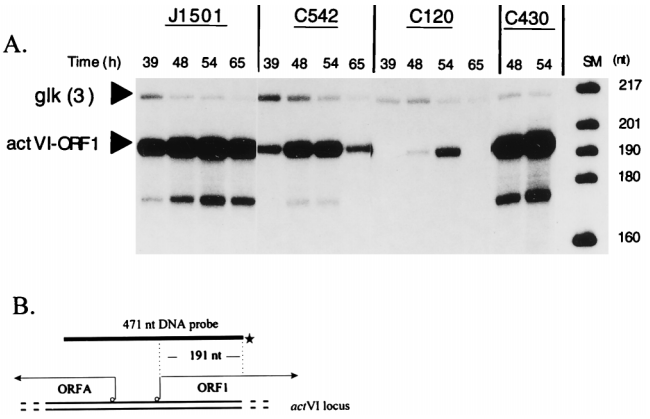

FIG. 3. Expression of *act*VI-ORF1 mRNA in J1501 (*abs*$^+$), C542 (*absA*), C120 (*absB*), and C430 (*absA* disruption). RNA was isolated from PGA plate-grown cultures at the times indicated and used for S1 nuclease protection analyses with a probe for the *act*VI-ORF1 transcript that should yield a protected fragment of 191 nt (22, 25). (A) The secondary bands below the *act*VI-ORF1 bands (approximately 167 nt), also obtained by other researchers using this probe (25), are of unknown origin; they may represent a secondary initiation site, a degradation product, or an artifact of the procedure. The *glk* probe was as described in the legend to Fig. 1. The size markers (SM) were as described for Fig. 1. (B) The uniquely end-labeled probe used for the *act*VI-ORF1 transcript is described in Materials and Methods. ORFA has been implicated in Act synthesis, but its role is unknown (22).

catalyzing an early reductive step in Act synthesis (22), and *act*I (ORF1 and ORF2), encoding components of the polyketide synthase that assembles the Act carbon backbone (21). The temporal expression of one of these, *act*VI-ORF1, has been well characterized in liquid culture, and the transcript is seen to accumulate as the culture enters stationary phase (25). In this work, transcript levels from *act*VI-ORF1 were monitored in the parental strain J1501 and in mutant strains C542 and C120. Expression of an *act*I transcriptional reporter gene fusion was also monitored.

In the experiment shown in Fig. 3A, the level of *act*VI-ORF1 transcript in the C542 (*absA*) mutant strain was reduced approximately sixfold over the time course of the experiment in comparison to J1501. Thus, the reduction in *act*VI-ORF1 transcript levels paralleled the reduction in *act*II-ORF4 transcript levels seen in the same time course of RNA isolation in Fig. 2A. In each of two similar assays of an independent RNA time course, *act*VI-ORF1 transcript levels were decreased more than eightfold in C542 (data not shown).

Figure 3A also shows that the levels of the *act*VI-ORF1 transcript were approximately 12-fold lower over the time course in the C120 (*absB*) strain than in J1501. In two similar assays of *act*VI-ORF1 transcript in an independent RNA time course, 12-fold- and 25-fold-lower amounts of *act*VI-ORF1 transcript were observed in C120 (data not shown).

To monitor transcription from another *act* promoter, *act*I, a *xylE* reporter gene fusion was used. The actinophage clone KC900 was used to produce a transcriptional fusion of *xylE* to the *act*I chromosomal promoter (see Materials and Methods). Fusions were constructed in the parental strain J1501 and in C542 (*absA*) and C120 (*absB*). J1501 strongly expressed the XylE product after 2 days of plate culture on R5 medium (5), while C542 and C120 did not visibly express XylE product during the 5-day time course (data not shown).

Regulation of expression of the Red-specific regulator *redD* by the *absA* and *absB* loci. Like Act production, production of Red is growth phase regulated, initiating in the transition phase of liquid-grown cultures and continuing in stationary phase. The regulation of Red production depends, at least in part, on the growth-phase-regulated expression of the Red antibiotic-specific regulator *redD*, which is seen to appear in the transition phase of liquid-grown cultures of *S. coelicolor* M145 (52). Figure 4 shows *redD* expression in a time course in which Red was visibly detectable at 48 h. Figure 4A shows that plate-grown cultures of J1501 also exhibit temporal regulation of *redD* and that the C542 (*absA*) culture exhibited approximately sevenfold-reduced levels of *redD* transcript. Figure 4A also shows that *redD* transcript abundance was reduced approximately sevenfold in C120 (*absB*). Transcripts encoding biosynthetic genes have not been characterized, and *red* biosynthetic gene transcription was not assessed in this study.

Effect of a disruption mutation of the *absA* locus on expression of *act*II-ORF4, *act*VI-ORF1, and *redD* transcripts. In contrast to the Abs$^-$ (antibiotic-deficient) phenotype of strain C542, another *absA* mutant strain, C430, displays a Pha phenotype (for precocious hyperproduction of antibiotics). Pha mutant strains such as C430 produce Act and Red 6 to 12 h earlier than J1501 and produce five- to eightfold-more Act and Red. This phenotype is associated with certain disruption mutations of the *absA* locus (5); strain C430 carries a disrupted *absA2* gene due to insertion of phage ϕC31 DNA (5).

In previous work, use of an *act*I::*xylE* chromosomal fusion to assess the expression of the *act*I biosynthetic gene in an *absA*-disrupted Pha strain demonstrated significant XylE activity 6 to 12 h earlier than in J1501, and the activity reached a fourfold-higher peak (5). In Fig. 2A, 3A, and 5, all representing experiments using RNA from the same time course, strain C430 was evaluated for expression of the *act*II-ORF4, *act*VI-ORF1, and *redD* transcripts, respectively. C430 expressed all of these transcripts at approximately twofold-higher levels than J1501 at the times shown. In this time course, Red was produced earlier than in the experiment shown in Fig. 4A, appearing by 30 h in J1501. *redD* transcript abundance has been seen to drop at later times in previous experiments (23) and here does so in both the J1501 and C430 strains.

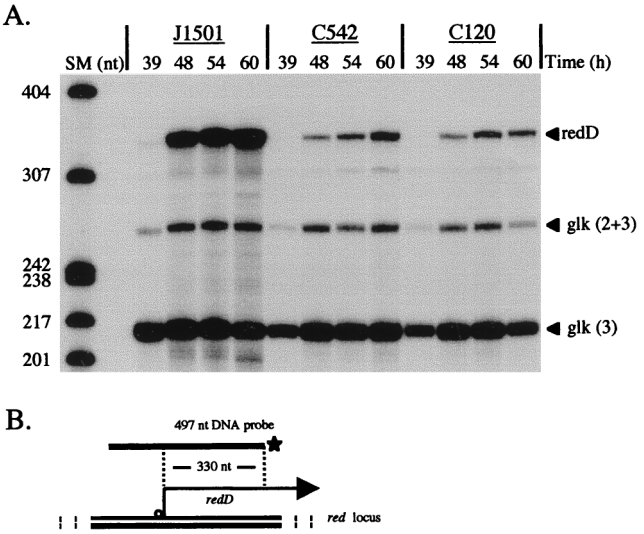

FIG. 4. Expression of *redD* mRNA in J1501 (*abs*$^+$), C542 (*absA*), and C120 (*absB*). RNA was isolated from PGA plate-grown cultures at the times indicated and used in S1 nuclease protection assays with a probe for the *redD* transcript that should yield a protected fragment of 330 nt (45, 52). (A) Bands labeled "*redD*" represent transcript from the *redD* gene. The *glk* probe was as described for Fig. 1. Size markers (SM) were as described for Fig. 1. (B) The uniquely end-labeled probe for the *redD* transcript is described in Materials and Methods.

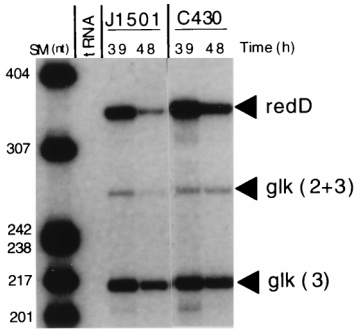

FIG. 5. Expression of *redD* mRNA in J1501 (*abs*$^+$) and C430 (*absA* disruption). RNA was isolated from PGA plate-grown cultures at the times indicated and used in S1 nuclease protection assays. The probes for *redD* and *glk* were as described for Fig. 4.

DISCUSSION

The results of this work indicate that the *absA* and *absB* gene products regulate expression of *act* and *red* antibiotic gene transcripts through regulation of the respective antibiotic-specific regulator genes. Previous work had established that *S. coelicolor* Act and Red production is limited by accumulation of sufficient quantities of the *act*II-ORF4- and *redD*-encoded regulators (25, 52). Hence, this work demonstrates that an important aspect of global regulation of antibiotic production is *absA*- and *absB*-mediated regulation of antibiotic pathway-specific regulators.

The visible phenotypes of both *absA* and *absB* Abs$^-$ strains were tight under the conditions of incubation used in these experiments, with little or no antibiotic produced after 5 days of incubation. However, the mutant effects exerted on the antibiotic gene transcripts were partial, with variability observed in different time courses, and so the amount of transcript accumulated in the Abs$^-$ strains was higher relative to that in J1501 than was the comparable amount of antibiotic detectably produced by these strains. One factor contributing to the amount of transcript seen in the C542 strain may be the presence in the cultures of a subpopulation of antibiotic-overproducing hyphae that result from *sab* (for suppressor of *abs*) suppressor mutations, which are spontaneously accumulating second-site suppressor mutations that restore antibiotic production. Such Act and Red pigment-overproducing *absA sab* mutants exist in a frequency high enough to cause extensive visible speckling of plate-grown cultures (46). It is not clear at this time whether the *absA* and *absB* mutations cause additional blocks to antibiotic production besides their effects on transcript levels as reported here, but the previous observation (10) that extra cloned copies of *act*II-ORF4 or *redD* are sufficient to restore Act or Red production, respectively, to the mutants suggests that reduced *act*II-ORF4 and *redD* expression is the critical limitation to antibiotic production.

Despite the Abs$^-$ mutants' effects on antibiotic transcript levels, it is noteworthy that the temporal profile of mRNA expression is not perturbed in the mutants. This observation would be compatible with a primary role for the *abs* gene products in maximizing antibiotic gene expression rather than in determining its timing. An alternative view of the data involving the Abs$^-$ *absA* strain is that the signals evident at later times reflect, at least in part, antibiotic production in the cultures' *sab*-suppressed subpopulation, as discussed above.

Although both Act and Red are subject to regulation by *absA* and *absB*, differences in the two antibiotics' temporal profiles are evident. For example, in the course of these studies, Red was visibly produced about 12 h earlier than Act in all time courses. However, the timing of the increases in *act*II-ORF4 transcript levels were similar to the increases of the *redD* transcript levels. Thus, the visible accumulation of Act lagged well behind the detection of *act* transcripts, whereas Red was detectable within hours of *redD* accumulation. Some studies have seen even greater lags between transcription and production (23); clearly, more factors responsible for the temporal profiles of these antibiotics remain to be elucidated.

In addition to their effects on the antibiotics Act and Red, both the *absA* and *absB* mutations also abolish production of the antibiotics Mmy and CDA (1, 2). Determination of the effects of *absA* and *absB* on *mmy* and *cda* regulation awaits characterization of the *mmy* and *cda* transcripts.

This work does not determine whether the *absA* and *absB* mutants' effects on message levels occur at the level of promoter usage or of transcript stability. Recent results (45) indicate that the *S. coelicolor absB* gene encodes a homolog of *E. coli* RNase III, a double-strand-specific RNase. It is tempting to speculate that an *absB*-encoded RNase III activity exerts control over antibiotic gene expression through posttranscriptional regulation of specific target genes. Definition of a structural or sequence motif recognized by *E. coli* RNase III has proven difficult (17, 43), so it is not currently feasible to use precedent from *E. coli* to predict whether potential RNase III targets are associated with the *act* and *red* genes studied here.

The *absA* locus encodes a putative negatively regulating two-component signal transduction system in which the *absA1* gene encodes a protein with similarity to the sensor-transmitter class and the adjacent *absA2* gene encodes a response regulator. It was the observation of an increase in antibiotics seen in *absA*-disrupted Pha strains that led to the hypothesis (5) that the *absA1A2* genes exert negative control over antibiotic gene expression. The increase in message levels seen in the C430 strain in this study lends support to this hypothesis. However, this work does not address the issue of whether AbsA2 acts directly as a repressor of *act*II-ORF4 and *redD* or whether the observed negative regulation involves additional proteins. It is also not known what signal AbsA1 senses.

Current evidence does not determine whether the *absA1A2* and *absB* genes function in the same or different pathways or whether they function in concert with or independently of other antibiotic regulators, such as *afsRKS*, *afsQ1Q2*, *abaA*, *abaB*, *cutRS*, and *relA* (4, 10, 12). Further analysis of the relationships of the *absA*- and *absB*-encoded products to these genes should provide significant information about the network of elements controlling antibiotic production.

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