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Nucleotide Sequence and Transcriptional Analysis of the *redD* Locus of *Streptomyces coelicolor* A3(2)

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Previous genetic evidence suggested that the *redD* gene product might be involved in the regulation of undecylprodigiosin (Red) biosynthesis in *Streptomyces coelicolor*. The *redD*$^+$ gene was subcloned on a 2.2-kilobase-pair restriction fragment from the *S. coelicolor redCD* region by complementation of *S. coelicolor* JF1 (*redD42*). The DNA sequence of the 2.2-kilobase-pair *redD*-complementing region was determined, and the *redD* coding sequence was identified by computer analysis and deletion subcloning. Transcription at the *redD* locus was analyzed by using in vivo promoter probing, high resolution S1 mapping, and in vitro runoff transcription. A face-to-face arrangement of promoters was deduced, in which the proposed *redD* promoter was opposed by a cluster of four other promoters for another unidentified open reading frame. In time course experiments, *redD* transcription preceded that at two biosynthetic loci, *redE* and *redBF*; transcription at the latter two loci was reduced in *redD42* mutants. The putative *redD* polypeptide lacked any strong sequence similarities to other known proteins.

Streptomycetes are free-living, mycelial, soil bacteria that differentiate both morphologically and physiologically during their life cycle (16). During idiophase, these microorganisms sporulate and produce an enormous array of secondary metabolites. The availability of molecular techniques applicable to *Streptomyces* species (17) has enabled detailed studies of gene regulation during secondary metabolism.

The red-pigmented antibiotic (Red) of *Streptomyces coelicolor* A3(2) is a mixture of tripyrroles closely related in structure to prodigiosin (35). The major components of Red are undecylprodigiosin and its cyclized derivative, butylcycloheptylprodiginine (32). Red is synthesized from amino acid and polyketide components as a secondary metabolite (5) during idiophase after primary cell growth has ceased (13). The system is an attractive model for use in studying the regulation of antibiotic production partly because Red and its yellow precursor, undecylnorprodigiosin, can be visually detected.

Biosynthesis of Red has been examined, using both genetic and molecular techniques. Biosynthetic mutants were isolated and ordered by cosynthesis (29), the entire gene cluster was cloned (13; F. Malpartida, unpublished data), and specific genes were localized by complementation of blocked mutants with plasmid subclones of *red* DNA (12, 13). These studies suggested that the *redD* gene product might participate in the regulation of Red biosynthesis for two reasons. First, *S. coelicolor* JF1 (*redD42*) failed to cosynthesize with any other *red* mutants (13, 29). Second, complementation of JF1 with plasmid clones of the 13-kilobase-pair (kbp) *redCD* region resulted in apparent Red overproduction.

Further examination of the *redD*$^+$ gene at the molecular level will contribute to the understanding of the regulation of antibiotic biosynthesis in *S. coelicolor*. In this study, we describe the nucleotide sequence and transcriptional analysis of the *S. coelicolor redD* locus.

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MATERIALS AND METHODS

Bacterial strains. The *Escherichia coli* strains used were JM103 and JM109 (36). The *S. coelicolor* strains used were BR140 (Red$^+$ *actVII140 hisA1 uraA1 strA1*) (29), JF1 (*redD42 actII177 argA1 guaA1*) (12), and M145 (17). The *Streptomyces lividans* strain used was TK54 (*his-2 leu-2 spc-1*) (18).

Plasmids and bacteriophages. *E. coli* plasmids used in this study were pGEM-5Zf(+) and pGEM-7Zf(+) (Promega Biotec). *Streptomyces* plasmids pIJ702 (20) and pIJ941 (21) were used as cloning vectors. The promoter probe vectors pCLL34 (a low-copy-number, SLP1.2-based vector) (11) and pIJ486/7 (high-copy-number, pIJ101-based vectors) (34) were used for detecting the promoter activity of cloned *Sau*3AI DNA fragments by the activation of promoterless brown pigment or aminoglycoside phosphotransferase genes, respectively, in *S. lividans* transformants. Plasmids pCLL35 and pCLL36 consist of a 422-bp *Sau*3AI insert containing the *redD*$^+$ promoter region cloned in pCLL34 or pIJ486, respectively. The *redD*$^+$ subclones, pCLL37, pCLL38, and pCLL40, are described below (see legend to Fig. 1). M13 phage derivatives mp18 and mp19 (36) were used for dideoxy DNA sequence analysis (31).

Media. *E. coli* strains were grown on LB medium (22) containing ampicillin at 100 μg/ml for primary selection or 50 μg/ml for growth. YT medium (36) was used for M13 propagation. The solid medium used for *Streptomyces* species was R2YE (17). Liquid cultures were grown in YEME plus 34% sucrose (17) for protoplast or plasmid preparations or in YEG (1% Difco yeast extract plus 1% glucose) for fermentation studies. Thiostrepton (kindly supplied by S. J. Lucania, E. R. Squibb & Sons, New Brunswick, N.J.) was used at 50 μg/ml in agar or 5 μg/ml in broth. Neomycin was used at 10 μg/ml in agar plates for promoter probe assays.

Enzymes. Restriction endonucleases, calf intestinal alkaline phosphatase, T4 polynucleotide kinase, and S1 nuclease were purchased from Boehringer Mannheim Biochemicals, New England BioLabs, Inc., or Bethesda Research Laboratories, Inc., and used as recommended by the supplier.

Recombinant DNA methods. Preparation of *Streptomyces* protoplasts, transformation, nucleic acid purification and

manipulation, and standard cloning procedures have been described elsewhere (17, 22).

DNA sequencing. DNA sequencing was performed by the dideoxy-chain termination method of Sanger et al. (31), using Sequenase (U.S. Biochemicals) according to the recommendations of the supplier. Overlapping sequence data were obtained by using a series of synthetic 17- to 20-base oligonucleotide primers made on an Applied Biosystems 380B synthesizer by beta-cyanoethylphosphoramidite chemistry. To reduce compressions, sequencing reactions were carried out at 42°C with 7-deaza-dGTP (Boehringer Mannheim) substituted for dGTP. Base-specific chemical cleavage of ^{32}P-end-labeled DNA fragments was as described by Maxam and Gilbert (23). DNA sequence analyses and comparison with the Protein Information Resource data bank were performed, using IntelliGenetics computer programs.

RNA isolation and filter hybridization. RNA was isolated from liquid cultures grown in YEG at the times indicated in the figure legends and blotted onto nitrocellulose as described elsewhere (17). Strand-specific RNA probes were synthesized on linearized pGEM subclones with either SP6 or T7 RNA polymerase according to protocols provided by Promega Biotec. Hybridization conditions were as described by Wahl et al. (33), except that dextran sulfate was omitted.

Densitometry. Autoradiograph dot blot hybridization signals in the linear range of film response were quantitated, using an LKB UltroScan XL.

S1 nuclease mapping. For probe preparation, the *redD*$^+$ promoter region was excised from pCLL35 on a 467-bp *Hin*dIII-*Sst*I fragment (see Fig. 2). This fragment was subcloned in pGEM-7Zf(+) for amplification in *E. coli*. *Hin*dIII-*Sst*I fragments were dephosphorylated with calf intestinal alkaline phosphatase and end labeled with [^{32}P]ATP and T4 kinase by standard techniques (22). Single-stranded, uniquely end-labeled probes were prepared by strand separation on polyacrylamide gels (23). Alternatively, single-stranded [^{32}P]CTP-labeled RNA probes were synthesized with either T7 or SP6 RNA polymerase on linearized pGEM-7Zf(+) templates containing the *redD* promoter region. RNA for S1 experiments was isolated from 2- to 3-day cultures of *S. lividans* harboring pCLL35 or pCLL36 that exhibited reporter gene activity or from *S. coelicolor*(pCLL38) at the time of Red production. Hybridization at 56°C and S1 digestions were done as described by Favaloro et al. (10).

In vitro transcription. RNA polymerase was isolated from mid-logarithmic-phase *S. coelicolor* M145 cultures as described by Buttner and Brown (6) and used in runoff transcription assays on gel-purified DNA templates as described by Buttner et al. (7). Dinucleotide-primed transcription experiments were performed at 30°C as described by Moran et al. (26).

Gel electrophoresis. DNA sequencing reactions, S1 nuclease digestion products, and runoff transcripts were electrophoresed on 6% polyacrylamide-7 M urea field gradient gels (1, 28, 30). DNA restriction fragments were excised from low-melting-point agarose gels in TBE buffer (22) and purified with Elutip-D columns (Schleicher & Schuell, Inc.) for use as transcription templates.

Antibiotic quantitation. Mycelium from 5-day liquid cultures was harvested by centrifugation and washed once each in 50 mM Tris hydrochloride (pH 7.5) and water. Red was extracted with acidified methanol, partitioned into chloroform, concentrated by vacuum evaporation, and suspended in methanol for spectroscopy. The amount of Red was calculated by setting a difference of 1.0 absorbance unit between A_{534} and A_{656} to be equivalent to 19.3 μg of prodigiosin (35).

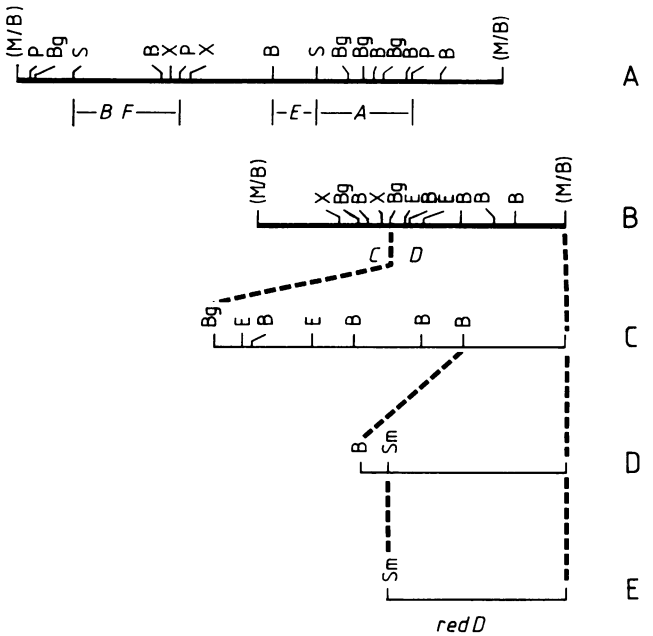


FIG. 1. Restriction maps of DNA cloned from the *red* gene cluster of *S. coelicolor*. (A) pIJ759, 21-kbp insert in pIJ922; (B) pIJ2342, 13-kbp insert in pIJ940; (C) pCLL37, 7.4-kbp insert in pIJ941; (D) pCLL38, 2.1-kbp insert in pIJ702; (E) pCLL40, 1.9-kbp insert in pIJ941. The locations of *red* genes A to F determined by complementation with plasmid subclones are indicated below the restriction maps. Dashed lines indicate subclones used to localize *redD*. Abbreviations: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; M, *Mbo*I; P, *Pst*I; S, *Sst*I; Sm, *Sma*I; X, *Xho*I.

RESULTS

Subcloning the *redD*$^+$ gene by complementation. The organization of *red* genes on two *Mbo*I partial restriction fragments of *S. coelicolor* DNA totaling about 34 kbp cloned in pIJ759 and pIJ2342 is depicted in Fig. 1. These plasmids complement previously described *red* mutations (13). Complementation studies indicated that the *redD* gene was located on the 13-kbp *Mbo*I partial restriction fragment insert cloned in pIJ2342 (F. Malpartida, unpublished data). The *redD* gene was subcloned to a 1.9-kbp *Sma*I-*Mbo*I fragment by restoration of Red production in strain JF1 (*redD42*) (Fig. 1).

Overexpression of Red by *redD*$^+$ transformants. A phenotypic consequence of transforming either Red$^+$ or *redD42* strains with plasmid subclones carrying the *redD*$^+$ gene is the visually detectable overproduction of Red on agar plates. Transformed colonies have a metallic red sheen on their surfaces, with delayed aerial mycelium formation and sporulation.

Overproduction of Red was independent of plasmid copy number. Liquid cultures of BR140 transformants carrying the high-copy-number *redD*$^+$ subclone pCLL38 produced approximately 31 times more Red than did BR140 carrying only the vector pIJ941 (Table 1). BR140 carrying the low-copy-number derivative pCLL40 also overproduced Red at similar levels. Transformants of JF1 (*redD42*) carrying pCLL38 produced about sixfold more Red than Red$^+$ control strains, whereas transformation of JF1 with pCLL40

TABLE 1. Levels of undecylprodigiosin production

Strain	Undecylprodigiosin (μg/mg of mycelium [dry wt])[a]
BR140(pIJ941) (control)	0.26
BR140(pCLL38)	7.61
BR140(pCLL40)	6.30
JF1(pIJ941) (control)	ND[b]
JF1(pCLL38)	1.44
JF1(pCLL40)	0.69

[a] Cultures (50 ml) were grown from 5% seed inocula in YEG at 30°C. The level of undecylprodigiosin was quantitated as described in Materials and Methods. Values represent the means of triplicate samples extracted from representative cultures.
[b] ND, None detected.

resulted in approximately threefold more production than that in wild-type controls.

Promoter probing. Previously, a collection of 41 promoter-active *Sau*3AI fragments from the 13-kbp *redCD* region were identified among *S. lividans* transformants by using the promoter probe vector pCLL34 (11). One of these clones, pCLL35, contained a 422-bp insert that hybridized to the 2.2-kbp *redD* region (data not shown). This insert was later shown to include the *redD* promoter region by DNA sequencing.

The 422-bp *redD* promoter insert exhibited bidirectional activity in two promoter probe vectors. *S. lividans* transformants produced brown pigments or were resistant to 10 μg of neomycin per ml when the *redD* promoter was cloned in both orientations relative to the reporter genes in pCLL34 or pIJ486/7, respectively. These results prompted us to characterize *redD* transcription in detail.

In vitro runoff transcription assays. To examine the apparent bidirectionality of the *redD* promoter region, a 467-bp *Hin*dIII-*Sst*I fragment from pCLL35 was subcloned in pGEM-7Zf(+) for amplification in *E. coli* and reisolated bearing either 5′ or 3′ extensions of 35 or 47 bp, respectively (Fig. 2). These overlapping restriction fragments were used as templates for *S. coelicolor* RNA polymerase in in vitro runoff transcription assays. Four transcription start sites were detected on the *Hin*dIII-*Sst*I template, yielding transcripts of approximately 400, 205, and 150 bp as well as a

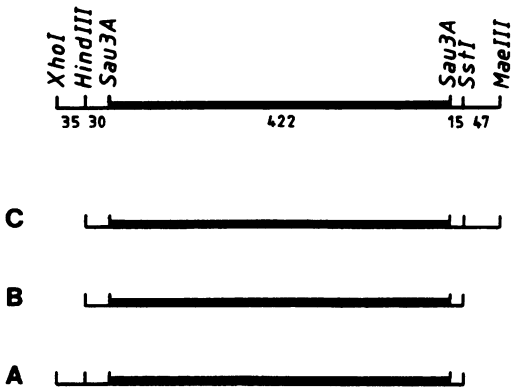


FIG. 2. Schematic representation of the promoter-active restriction fragments used as templates for *S. coelicolor* RNA polymerase. Pertinent restriction sites of the *redD* promoter templates bearing pGEM-7Zf(+) polylinker extensions are shown (top). Numbers indicate base pairs. Templates used to generate the runoff transcripts were *Xho*I-*Sst*I (A), *Hin*dIII-*Sst*I (B), and *Hin*dIII-*Mae*III (C).

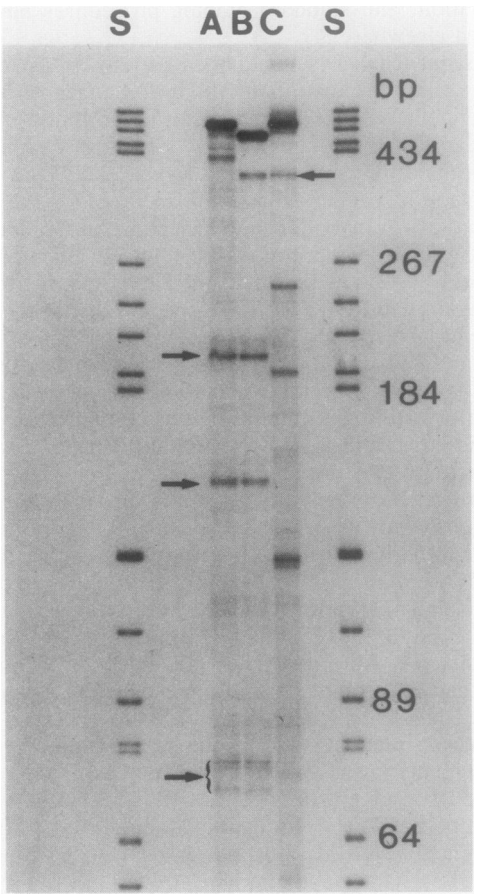


FIG. 3. In vitro transcription of the *redD* locus promoters. Transcripts and relative directions of transcription are indicated by arrows. Runoff transcripts were generated from *Xho*I-*Sst*I (lane A), *Hin*dIII-*Sst*I (lane B), or *Hin*dIII-*Mae*III (lane C) restriction fragments. Heat-denatured, ^{32}P-labeled *Hae*III fragments of pBR322 (lanes S) are also shown.

triplet of 70, 74, and 75 bp (Fig. 3, lane B). A template extension 47 bp beyond *Sst*I to the *Mae*III site resulted in longer transcripts corresponding closely to the sizes predicted from three of the start sites (252, 197, and 122 bp, respectively) (Fig. 3, lane C). A template extension 35 bp in the opposite direction to *Xho*I yielded extended transcripts from the fourth site of approximately 435 bp (Fig. 3, lane A). These results suggested a bidirectional, overlapping (face-to-face) arrangement of promoters in which transcription from three rightward start sites was opposed by transcription from a single leftward start site.

High-resolution S1 nuclease mapping. S1 nuclease mapping experiments (Fig. 4) were used to identify the precise location of transcription start sites initiated from the promoters identified in vitro. Single-stranded *Hin*dIII-*Sst*I probes labeled at their 5′ ends were hybridized with RNA isolated from *S. lividans* carrying the 467-bp *Hin*dIII-*Sst*I *redD* promoter subclone in either low-copy-number (pCLL34) (Fig. 4, lane B) or multicopy (pIJ486) (Fig. 4, lane C) vectors and were digested with S1 nuclease. The three rightward transcripts observed in runoff assays as well as a fourth rightward transcript of 110 bp not seen in vitro were detected in these experiments. These promoter start sites were designated Pr1 to Pr4. Interestingly, Pr1 transcripts initiated at

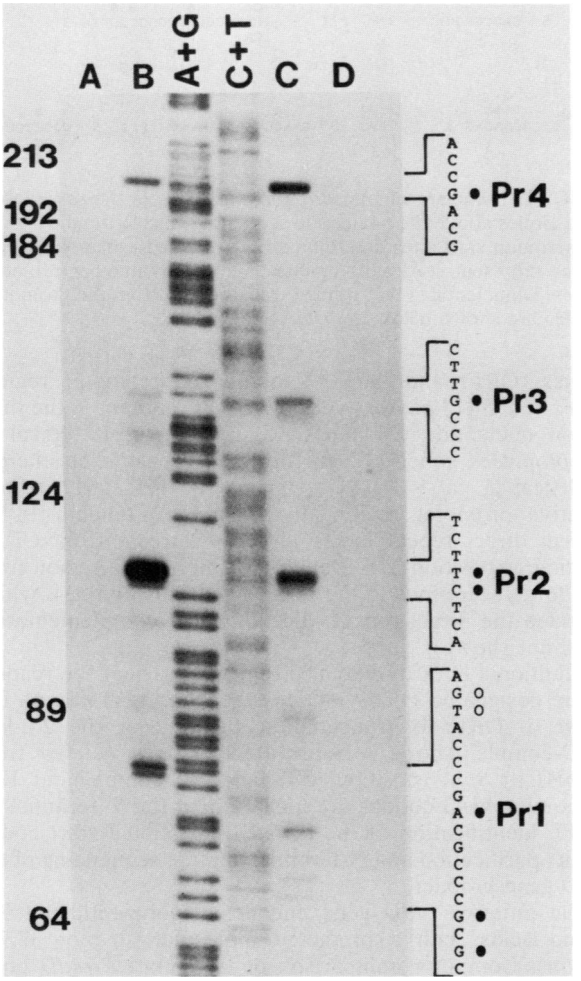


FIG. 4. High-resolution S1 nuclease mapping of transcription initiation sites, using DNA restriction fragment B (Fig. 2) as the probe. Prior to S1 digestion, 5'-terminal-^{32}P-end-labeled strand-specific DNA probes were hybridized with 40 μg of RNA from *S. lividans* TK54 containing pCLL34 (lane A), pCLL35 (lane B), pCLL36 (lane C), or pIJ486 (lane D). A+G and C+T are Maxam-Gilbert DNA sequence ladders of the probe. The DNA sequences near the transcription start sites are shown. Solid circles represent the most probable transcription start sites. Open circles indicate Pr1 start sites unique to low-copy-number promoter clones. Numbers indicate sizes of heat-denatured standards (bases).

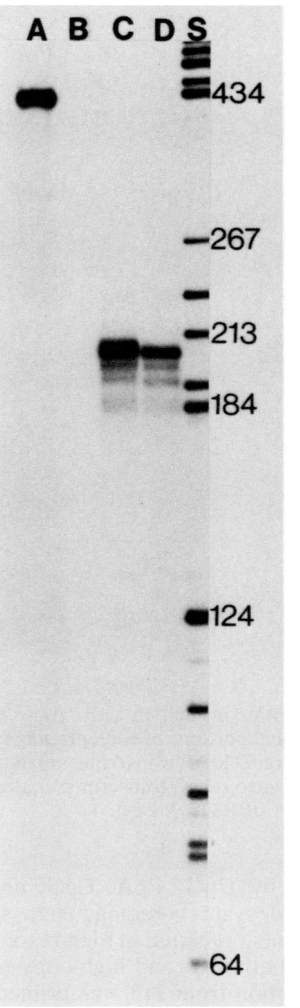


FIG. 5. High-resolution S1 nuclease mapping using ^{32}P-labeled RNA probes complementary to the *S. coelicolor Sau*3AI fragment containing the *redD* locus promoters. ORFr-specific probe hybridizations and digestions included 40 μg of RNA from BR140(pCLL38) without S1 nuclease (lane A), 40 μg of tRNA plus 200 U of S1 nuclease (lane B), 40 μg of RNA from BR140(pCLL38) plus 200 U of S1 nuclease (lane C), or 40 μg of RNA from BR140(pCLL38) plus 600 U of S1 nuclease (lane D). S, Denatured ^{32}P-labeled *Hae*III fragments of pBR322. Note that the size of the probe in lane A (391 bases) is the sum of the 341-base *Sau*3AI fragment plus 50 bases from the pGEM-7ZF(+) polylinker.

multiple sites and were 5 bases longer in low-copy-number promoter clones.

DNA sequence data subsequently revealed that the 422-bp promoter clone used in initial transcription studies contained additional sequences unrelated to the *redD* locus beginning 16 bp upstream from Pr4. Therefore, the 341-bp *Sau*3AI fragment identified by DNA sequencing to contain the *redD* promoter region was subcloned into pGEM-7Zf(+) for synthesis of ^{32}P-labeled riboprobes. S1 nuclease assays with RNA isolated from *S. coelicolor* BR140(pCLL38) and strand-specific RNA probes demonstrated transcription initiation primarily at Pr4 (Fig. 5) and, upon longer exposure of the autoradiograph, from the other rightward start sites (data not shown). No additional transcription initiation sites were identified. Thus, although the origin of the extraneous sequences upstream from the promoter cluster in the original 422-bp *Sau*3AI fragment is unknown, the transcription initiation sites were unaffected.

We were unable to detect the putative leftward transcription start site (Pl1) in vivo with either end-labeled DNA probes or RNA probes uniformly labeled with [^{32}P]CTP. However, strand-specific RNA probes complementary to the 168-bp *Sph*I-*Cla*I restriction fragment internal to open reading frame 1 (ORFl) indicated that transcription through this region proceeds in the same leftward direction as transcription from Pl1 (data not shown).

Dinucleotide-primed transcription. To demonstrate that the transcription start sites identified in S1 assays resulted from RNA polymerase initiation at unique points and were not RNA processing or degradation artifacts, purified *S. coelicolor* RNA polymerase was primed with dinucleotides on 467-bp *Hin*dIII-*Sst*I templates. Dinucleotides are known to prime transcription at or near the sites of transcription to which they are homologous (7, 9, 15, 24). Transcription from

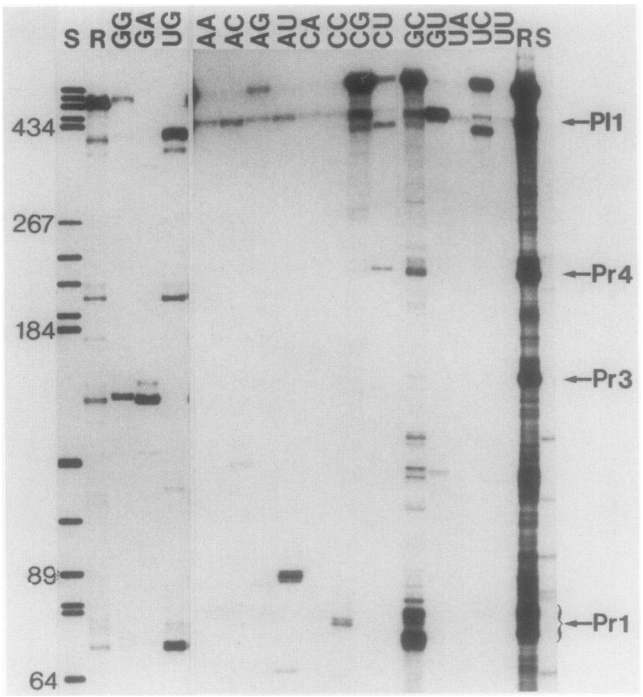


FIG. 6. Dinucleotide-primed in vitro transcription, using template B (Fig. 2). Equal portions of each priming reaction are shown. The position of the *redD* locus transcripts are indicated with arrows. R, Conventional in vitro runoff transcripts; S, denatured ^{32}P-labeled *Hae*III fragments of pBR322 (bases).

Pr1 was primed by UpG, CpA, CpG, and GpC (Fig. 6). These dinucleotides are homologous to sequences at the multiple start points predicted in high-resolution S1 mapping experiments for both low- and high-copy-number promoter clones. Transcription from Pr3 was primed by GpG, GpA, CpG, and, unexpectedly, GpC. Other unexpected transcripts primed by GpC were also observed, and it is possible that these arose from illegitimate priming as described by Buttner et al. (7). Transcription from Pr4 was primed by UpG, CpU, and GpC. These results verified those obtained from S1 nuclease assays for rightward transcription at Pr1, Pr3, and Pr4 and strongly suggest that the observed transcripts are not RNA processing artifacts. As in previous experiments, transcription from Pr2 was not detected in vitro.

Interestingly, leftward transcription from Pl1 appeared to be primed to some degree by all dinucleotide combinations (Fig. 6). Very strong priming occurred with UpG, CpG, GpC, and GpU. The sequence 5′-CGC-3′ occurs near the 5′ terminus of Pl1 transcripts estimated by size, and 5′-UG-3′ occurs 6 bases upstream from this point. In the absence of S1 mapping data, position 1689 is tentatively considered the Pl1 transcription start site. An alternative interpretation of these results is that these transcripts represent rightward transcription initiated at the template end that terminated at the inverted repeat containing Pl1 (see Fig. 8). However, in this case one would expect transcripts of equal intensity for all dinucleotide-primed transcription reactions.

A comparative summary of in vivo and in vitro transcription data is presented in Fig. 7.

DNA sequence of the *redD* locus. The DNA sequence of the 2.2-kbp *redD*$^+$ region of *S. coelicolor* is shown in Fig. 8. Computer analysis revealed two large potential open reading

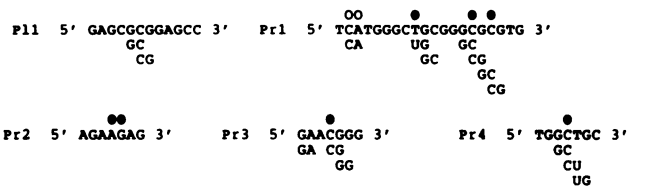


FIG. 7. Summary of transcription start sites in the *redD* locus. Solid circles above the nucleotide sequence indicate the most likely transcription start sites determined by S1 nuclease mapping. Open circles represent start sites unique to low-copy-number promoter clones. Dinucleotides that primed transcription from the promoters in vitro are shown below the DNA sequence.

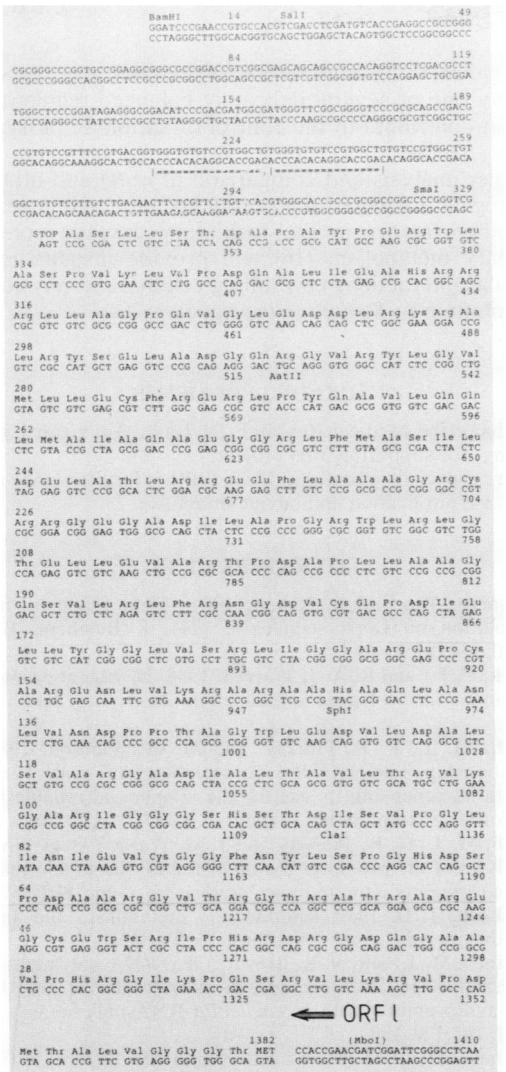
frames, ORFr and ORFl, flanking the promoter region. ORFr and ORFl possess very high G+C content in the third codon nucleotide (98 and 89%, respectively), typical of *Streptomyces* genes (3). An inverted repeat (free energy, −28 kcal [1 cal = 4.184 J]) spans the Pl1 start site and putative promoter region. In addition, a tandem 18-bp, perfect direct repeat exists just downstream from ORFl, starting at position 211. Deletion of the *redD*$^+$ region from the *Bam*HI terminus to the *Sma*I site at position 324, which included the direct repeat, did not affect complementation (data not shown).

Additional lines of evidence that suggest that the reading frame designated ORFl encodes the *redD* gene are as follows. (i) ORFr is truncated at the end of the 2.2-kbp *redD*-complementing region. (ii) Subclones deleted from *Bam*HI to *Sph*I (position 954) failed to complement JF1. Since two AUG codons are located near the 5′ terminus of ORFl, identification of the actual translational start codon awaits purification and N-terminal peptide sequencing of the *redD* gene product.

The putative *redD* gene encodes a polypeptide of 350 amino acids with a predicted molecular size of 37,794 daltons. Computer comparison of the putative *redD* polypeptide sequence with other known proteins in the Protein Information Resource data base failed to identify any significant similarities.

Temporal expression of ORFl and ORFr transcripts. Since ORFl and ORFr are divergently arranged with opposing promoters, the temporal expression of these transcription units was examined. Dot blot analyses (Fig. 9) with strand-specific RNA probes complementary to the 341-bp *Sau*3AI promoter region showed that (i) wild-type transcription in both directions reached peak levels 60 h after the start of fermentation of *S. coelicolor* BR140 in YEG medium, prior to the production of Red; (ii) ORFr transcription followed a similar temporal pattern in strain JF1, but a peak for OrRFl transcription was not observed; and (iii) ORFr message levels were greater than those of ORFl in both *red*$^+$ and *redD42* strains. Any temporal differences between right- and leftward transcription were beyond the resolution of these experiments. A noteworthy observation was that ORFl (*redD*) transcription preceded that of *redE* and *redBF* (see Fig. 10), consistent with the proposed regulatory role for *redD* in *red* gene expression.

***red* gene expression in *redD42* mutants.** Previously, it was noted that (i) both *redE* and *redF* gene products were required for wild-type levels of the O-methyltransferase involved in Red biosynthesis and (ii) *redD* mutants had no detectable O-methyltransferase activity (13). To determine whether the *redD* gene product affects transcription of these *red* genes, transcription at the *redE* and *redBF* loci was compared for *S. coelicolor* BR140 (*actVII140* Red$^+$) and JF1



(*actII177 redD42*) during the course of fermentation in YEG (Fig. 10). The *redE* message level in total RNA was assayed with strand-specific RNA probes synthesized on a 1.79-kbp DNA fragment that complemented *redE60* mutants (12). Transcription from PL2-7b, a 79-bp, developmentally regulated promoter from the *redBF* region (unpublished data), was also assayed with complementary RNA probes. Transcription of the *redE* gene peaked just prior to the appearance of Red in strain BR140. We were unable to explain the relatively high signal observed for *redE* transcription at 36 h. Densitometric scanning indicated that *redE* message levels were reduced as much as 3.5-fold below that of the wild type at 84 h in strain JF1. PL2-7b transcription was also detected prior to Red production at 84 h but reached maximum levels at 132 h postinoculation. At that time, PL2-7b message levels in the *redD42* mutant strain JF1 were one-third that of the wild type. Strain JF1 does not appear to be generally deficient in transcriptional capability, since ORFr transcription in this strain was similar to that for strain BR140 (Fig. 9). Thus, the *redD* gene product appears to be necessary for wild-type levels of the other *red* biosynthetic genes studied.

DISCUSSION

In this paper, we report the DNA sequence and transcriptional analysis of the *redD* locus of *S. coelicolor* A3(2). Interest in the *redD* gene stems from previous genetic studies that implicated *redD* in the regulation of undecylprodigiosin (Red) biosynthesis in *S. coelicolor* (13, 29).

The *redD* locus possesses a face-to-face divergent promoter arrangement in which the putative *redD* promoter is oriented opposite a cluster of four closely spaced promoters for an unidentified open reading frame. The presence of divergent genes transcribed from promoters in close proximity has emerged as a common form of gene organization (for a review, see reference 2). Among other streptomycetes, the erythromycin resistance (*ermE*) gene of *Streptomyces erythraeus* (4, 19), the aminoglycoside phosphotransferase (*aph*) gene of *Streptomyces fradiae* (4, 19), and the adjacent streptomycin genes *strR* and *strD* of *Streptomyces griseus* (8) are components of divergent gene sets with bidirectional promoter regions.

The DNA sequences upstream from the transcription initiation sites Pr1 and Pr4 resemble AT-rich −10 promoter regions (14). However, similar sequences were not apparent for the other transcription start sites in the *redD* locus. Comparison of the promoter sequences for the *redD* gene (ORFl) and the streptomycin regulatory gene *strR* (8) revealed 70% homology in the regions of −11 to −37 from the transcription start sites (data not shown). It is tempting to speculate that these regions include DNA sequences important for programming the expression of these regulatory genes during differentiation.

The face-to-face gene configuration described here for the genes at the *redD* locus could result in divergent, partially overlapping transcription which might provide a means of

FIG. 8. DNA sequence of the *redD* locus. Arrows indicate the location of the promoter cluster and relative directions of transcription. The positions of the characterized transcription start sites in the intergenic region between ORFl and ORFr are indicated. Circles represent the most likely transcription start sites. Putative −10 promoter sequences (14) are overscored. Direct or inverted repeats are indicated by double dashed lines. The nucleotide and putative polypeptide sequences are numbered; they have been deposited in GenBank and assigned accession no. M29790.

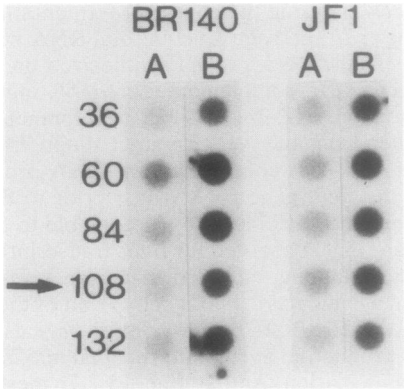


FIG. 9. Temporal transcription of *redD*. Seed cultures were grown from spores in 50 ml of YEG at 30°C with shaking at 200 rpm for 36 h. A portion (30 ml) was inoculated into 600 ml of YEG and grown under identical conditions. Samples (100 ml each) were removed at the indicated times, harvested by centrifugation, and divided for RNA extraction and Red quantitation. Total RNA (5 μg) was spotted onto nitrocellulose and hybridized with strand-specific RNA probes complementary to the promoter region of ORFl (A) or ORFr (B). Numbers indicate fermentation time (hours). The arrow indicates the time of Red production.

fine-tuning *redD* expression and, consequently, control of Red biosynthesis. Interesting models of control include interference between oppositely directed polymerase molecules or translational inhibition by the formation of partially duplex mRNA molecules (25, 27). In vivo heteroduplex mRNA formation is also one possible explanation for our inability to define the 5′ end of the *redD* transcript by S1 nuclease mapping.

Mechanistically, little is currently known in general about developmental programs that control antibiotic biosynthesis during secondary metabolism. We have identified the putative *redD* coding sequence and found no apparent similarity between the predicted polypeptide and other known regulatory proteins in the Protein Information Resource data base. The dramatic increase in Red production by low-copy-number clones of *redD* is especially intriguing in light of the slight effect on transcription observed for two Red biosynthetic genes, *redE* and *redBF*. The *redD* gene product is probably one of several delicately balanced regulatory factors that contribute to the control of antibiotic biosynthesis in *S. coelicolor*.

Further analysis and comparison of *redD* and other antibiotic regulatory genes, their products, and their interactions with other factors in the regulatory cascade controlling secondary metabolism (16) will provide insights into the molecular interactions controlling cellular and physiological differentiation in *Streptomyces* species.

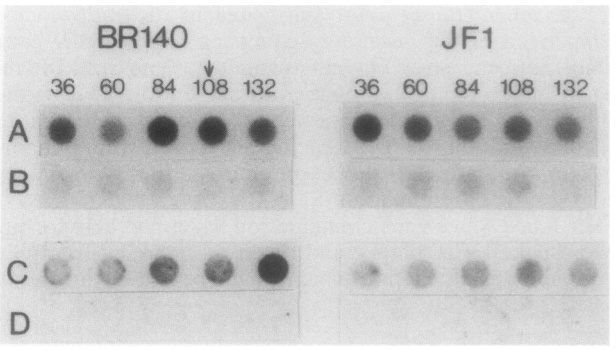


FIG. 10. Temporal expression of *redE* and *redBF* PL2-7b. RNA from *S. coelicolor* BR140 or JF1 was extracted from culture samples harvested during the course of fermentation in YEG (as described in the legend to Fig. 9) at the indicated times. RNA (2 μg) was spotted on nitrocellulose and hybridized with the following strand-specific runoff RNA probes: (A) antisense *redE*; (B) sense *redE*; (C) antisense PL2-7b; (D) sense PL2-7b. The arrow indicates the time of Red production. Note that the relatively high signal for *redE* at 36 h was reproducible for both strains.

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