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SCO5745, a Bifunctional RNase J Ortholog, Affects Antibiotic Production in *Streptomyces coelicolor*

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The bacterial RNases J are considered bifunctional RNases possessing both endo- and exonucleolytic activities. We have isolated an RNase J ortholog from *Streptomyces coelicolor* encoded by the gene *sco5745*. We overexpressed a decahistidine-tagged version of SCO5745 and purified the overexpressed protein by immobilized metal ion affinity chromatography. We demonstrated the presence of both 5′-to-3′ exonucleolytic and endonucleolytic activities on the *Bacillus subtilis thrS* transcript. Exonucleolytic activity predominated with 5′ monophosphorylated *thrS*, while endonucleolytic activity predominated with 5′ triphosphorylated *thrS*. While *sco5745* is the only RNase J allele in *S. coelicolor*, the gene is not essential. Its disruption resulted in delayed production of the antibiotic actinorhodin, overproduction of undecylprodigiosin, and diminished production of the calcium-dependent antibiotic, in comparison with the parental strain.

The decay of mRNA and the concomitant regulation of gene expression at this level are controlled by a number of key RNases present in a variety of combinations in both Gram-negative and Gram-positive bacteria. There is growing appreciation of how evolutionarily distant bacteria functionally combine quite different sets of proteins in the regulation of RNA metabolism (1, 2). RNase J was the first of two bacterial RNases discovered to possess both exo- and endonucleolytic activities. RNase BN acts as a 3′-to-5′ distributive exonuclease releasing mononucleotides and producing a ladder of digestion products. Cleaving endonucleolytically, it produces short 3′-end fragments. While active on single-stranded substrates, RNase BN prefers duplexed substrates and is inhibited by the presence of a 3′-end phosphoryl group and 3′-CCA sequence (3).

Originally characterized by two alleles in *Bacillus subtilis* (4), RNase J is widely distributed in other Gram-positive species as a single allele, as in *Mycobacterium smegmatis* (5), and as two alleles in *Streptococcus pyogenes* (6). RNases J have also been characterized in the Gram-negative bacterium *Sinorhizobium* (7), in the hyperthermophilic archaea *Pyrococcus abyssi* and *Thermococcus kodakaraensis* (8), and in plant chloroplasts (9). *B. subtilis* expresses two paralogous proteins, RNase J1 and J2. The two proteins possess endo- and exonuclease activities in *vitro* but are not equally active. Recently, it has been argued that the major function of RNase J1 is as an exonuclease, and no specific function has been assigned to RNase J2. However, the two proteins form a heterotrimer in *vitro* that cleaves a model substrate at a different site than the J1 homodimer (10). The single-stranded exonuclease activity of RNases J appears to be strongly dependent upon the phosphor- ylation state of the 5′ end of transcripts, usually showing a distinct preference for the monophosphorylated state (4, 9, 11, 12).

*Streptomyces* are Gram-positive actinomycetes noted for their production of antibiotics, antifungals, and chemotherapeutic drugs (13). *Streptomyces coelicolor* is a model for the study of the regulation of antibiotic biosynthesis. Regulation occurs at multiple levels, from specific antibiotic pathways to the global control of multiple antibiotics (14). Because of our ongoing interest in mechanisms of RNA decay in *Streptomyces*, and their relationship to antibiotic production, we have undertaken the identification, isolation, and characterization of the *S. coelicolor* RNase J ortholog, SCO5745. We examined the activity of the purified enzyme by utilizing as a substrate the upstream leader region of the *thrS* gene from *B. subtilis*, a well characterized RNase J substrate (15, 16). We show that SCO5745 is active both as an exonuclease and as an endonuclease. Cleaving *thrS* internally at many sites, SCO5745 produces a ladder of short products located proximal to the 5′ end, lending support to the hypothesis proposed by Taverniti et al. (5) that RNase J uses a sliding mechanism for endonucleolytic cleavage of triphosphorylated substrates. We also demonstrate that *sco5745* is not essential and that disruption of the gene affects antibiotic production in the disrupted strain.

MATERIALS AND METHODS

**Bacterial strains.** *Escherichia coli* strains DH5α and Rosetta 2(DE3)pLysS (Novagen) were used as hosts for plasmid manipulation and protein overexpression, respectively. *E. coli* strains BW25113 and ET12567 (dam dem hisD) were used for gene disruption and conjugation purposes (17). *E. coli* strains were grown on Difco nutrient agar or Luria-Bertani medium supplemented with carbenicillin (100 μg ml⁻¹) and/or kanamycin (50 μg ml⁻¹), as necessary. *S. coelicolor* M145 and JSE2301 were grown as necessary on soy flour-mannitol (SFM) agar to obtain spores, on R2YE agar, or in liquid medium (18) with viomycin (30 μg ml⁻¹), nalidixic acid (25 μg ml⁻¹), or apramycin (50 μg ml⁻¹). *Streptomyces* minimal medium containing carboxymethyl cellulose as a dispersant (18, 19) was used for liquid cultures. Spores were pregerminated as described previously (20), and growth of cultures was monitored by measuring the absorbance at 450 nm (A₄₅₀).

**Construction of the SCO5745 disruptant JSE2301.** To disrupt *sco5745*, the coding sequence was replaced by a viomycin resistance cassette using the REDIRECT λ Red-mediated recombination system for *Streptomyces* (21). Thus, cosmid SC9A10, which bears *sco5745*, was introduced by electroporation into *E. coli* strain BW25113 containing the λ Red
plasmid. The viomycin resistance cassette was excised from pJ780 (18, 21) with EcoRI and HindIII. The cassette was used as a template for PCR and amplified using primers Sco5745RD-F1 and Sco5745RD-R1 (Table 1). The strain carrying both cosmids SC9A10 and the λ Red plasmid was electrotransformed with the extended viomycin resistance cassette. Transformants were plated on Difco nutrient agar containing carbenicillin (100 μg ml⁻¹), kanamycin (50 μg ml⁻¹), and viomycin (50 μg ml⁻¹). Larger colonies were restreaked on plates with selective antibiotics (carbenicillin, kanamycin, viomycin) to enrich for cells bearing the mutant cosmids. Colonies were screened by PCR using primers 5745OutF1 and 5745OutR1 (Table 1). These primers are situated approximately 100 bp upstream and downstream, respectively, of the deleted SCO5745 open reading frame (ORF) and produced a 1,856-bp fragment if SCO5745 had been replaced by the viomycin resistance cassette. Cosmid DNA from disrupted transformants was electroporated into the methylation-deficient E. coli host strain ET12567 containing the nontransmissible plasmid pUZ8002 (18, 21). The marked cosmids were transferred to S. coelicolor M145 by conjugation with the E. coli strain, Desired S. coelicolor exconjugants (SCO5745 negative; Vio¹ Kan¹) were selected by restreaking single colonies on R2YE plates with and without kanamycin, and the identity of positive exconjugants was verified by PCR using primers 5745OutF1 and 5745OutR1 (Table 1). The strain bearing the disrupted SCO5745 was designated JSE2301.

Two strains (JSE2301/pSE2255 and JSE2301/pJB8600) were constructed for antibiotic production studies. The plasmids pSE2255 and pJB8600 (see "Plasmid construction" below) were electroporated into E. coli ET12567/pUZ8002, and transformants were conjugated with JSE2301. Integration of either plasmid into the JSE2301 chromosome was confirmed by selecting for viomycin- and apramycin-resistant exconjugants and via PCR using primers SCRNJF1 and SCRNJR1 (Table 1).

**Plasmid construction.** The sco5745 gene was PCR amplified using the Expand high-fidelity PCR system (Roche) and 50 ng of S. coelicolor M145 chromosomal DNA as the template. A forward primer, SCRNJF1 (Table 1), introduced an NdeI site overlapping the transcription start site. A reverse primer, SCRNJR1 (Table 1), introduced a BamHI site immediately downstream of the transcription stop codon. The PCR product was cloned into plasmid pCR2.1-TOPO in accordance with the manufacturer’s protocol (Invitrogen) and designated pSE2200. The ca. 1,700-bp NdeI-BamHI fragment was excised from pSE2200 and directionally cloned into the overexpression plasmid, pET-19b (Novagen), which carries an N-terminal leader transcript. Oligonucleotide primers used in this study

<table>
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</tr>
<tr>
<td>5745OutF1</td>
<td>5'-GCT CCA GGT TGG CGC TGT CGG TGG GCC GAC</td>
</tr>
<tr>
<td>5745OutR1</td>
<td>5'-AGG GGA GAT GTA AAC GTA CTG</td>
</tr>
<tr>
<td>SCRNJR1</td>
<td>5'-GGT GAG GAG AGT CAT ATG AGT CAT CCG</td>
</tr>
<tr>
<td>SCRNJF1</td>
<td>5'-CGG TGG GCC GAC GCA TCC</td>
</tr>
<tr>
<td>PBHP128</td>
<td>5'-AGA ATT CTA ATA CGA CTC ACT ATA AGG AGA</td>
</tr>
<tr>
<td>PBHB129</td>
<td>5'-TAT CTA GAT ATC TTC TGT TGT TCC</td>
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**Underlined sequences indicate restriction enzyme cleavage sites NdeI, BamHI, EcoRI, and PstI.**

Assay of SCO5745 activity. Sixteen-microliter assay mixtures contained 1× New England BioLabs restriction buffer 3 (100 mM NaCl, 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM dithiothreitol [DTT], pH 7.9), typically with 1.2 μg SCO5745 (ca. 1 μM) and 1 pmol [³²P]GTP (6,000 Ci/mmol; PerkinElmer). The specific activities of the 5'-monophosphorylated and 5'-triphosphorylated substrates were equalized (ca. 4,000 cpm/pmol) by mixing them with unlabelled transcript. Internally labeled substrates were transcribed in vitro using T7 RNA polymerase (Promega) in accordance with the manufacturer’s protocol and incorporated [α-³²P]GTP (3,000 Ci/mmol; PerkinElmer). Following in vitro transcription, unincorporated label was removed either by use of a NucAway spin column (Ambion) or RNAs were purified from 1% low-melting-point agarose gels.

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**Preparation of RNA substrates.** Templates for in vitro transcription were created by linearizing plasmid pSE2250 with XbaI to produce a ca. 384-base thRS transcript. Unlabeled, large-scale transcription reactions were performed using the Megascript T7 high-yield transcription kit (Ambion) according to the manufacturer’s protocol, and transcripts were subsequently either 5’ or 3’ end labeled. For 5’-end labeling, transcripts were treated with calf intestinal alkaline phosphatase (CIP), followed by T4 polynucleotide kinase (PNK; Promega) and [γ-³²P]ATP (3,000 Ci/ mmol; GE Healthcare). For synthesizing 3’-end-labeled substrates, the 5’ end was first converted to 5’-P by treatment with CIP and T4 PNK. Subsequently, 5’-³²P-labeled cytidine 3’-5’-bis(phosphate) (cCP) was ligated to the 3’ end with T4 RNA ligase (Promega) and incubated overnight at 14°C. For 5’-end triphosphate labeling, the Megascript T7 high-yield reaction mixture was modified by reducing the GTP concentration from 75 mM to 15 mM and including 70 μCi of [γ-³²P]GTP (6,000 Ci/mmol; PerkinElmer). The specific activities of the 5’-monophosphorylated and 5’-triphosphorylated substrates were equalized (ca. 4,000 cpm/pmol) by mixing them with unlabeled transcript. Internally labeled substrates were transcribed in vitro using T7 RNA polymerase (Promega) in accordance with the manufacturer’s protocol and incorporated [α-³²P]GTP (3,000 Ci/mmol; PerkinElmer). Following in vitro transcription, unincorporated label was removed either by use of a NucAway spin column (Ambion) or RNAs were purified from 1% low-melting-point agarose gels.

**Overexpression and purification of SCO5745.** Overexpression of the SCO5745 protein was performed in Rosetta 2(DE3) pLysS cells (Novagen) carrying plasmid pSE2201. A 500-ml culture of Luria-Bertani medium containing 34 μg ml⁻¹ chloramphenicol and 50 μg ml⁻¹ carbenicillin was induced with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) when the A₆₀₀ reached 0.5 to 0.7 and grown for three additional hours. Cells were harvested by centrifugation, the pellet was stored overnight at −20°C, and cells were disrupted using a French press (10,000 lb/in²) in lysis buffer: 50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 20 mM imidazole, 5% glycerol, 5 mM β-mercaptoethanol, 2 mM PMSF (phenylmethylsulfonyl fluoride), and Complete EDTA-free protease inhibitor cocktail (Roche). The resulting suspension was centrifuged for 20 min at 13,000 × g, and the supernatant was applied to a 5-ml HisTrap HP column (GE Healthcare) and eluted stepwise in lysis buffer containing 50, 100, 150, and 200 mM imidazole. Fractions were analyzed by 12.5% lithium dodecyl sulfate (LDS)-PAGE analysis, and those containing SCO5745, free of contaminating proteins, were pooled. Pooled fractions were dialyzed against storage buffer, i.e., 300 mM NaCl, 50 mM NaPO₄ [pH 8], 5 mM MgCl₂, and 10% glycerol, and stored at −80°C.

**TABLE 1 Primers used in this study**

<table>
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</tr>
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**Underlined sequences indicate restriction enzyme cleavage sites NdeI, BamHI, EcoRI, and PstI.**
The calcium-dependent antibiotic (CDA) assay essentially followed that described by Yang et al. (24). Inocula of 10⁹ spores of M145, JSE2301, JSE2301/pJSE2255, and JSE2301/pIJ8600 were plated on Oxoid nutrient agar (10 ml) and incubated at 30°C for 3 days. The plates were then frozen at −80°C overnight and slowly thawed to room temperature. Mycelium and medium were centrifuged (13,000 g, 10 min), and the resulting supernatant was collected for assay of CDA production. CDA-sensitive B. subtilis BG267 was used as an indicator strain. For each strain, a 75-μl aliquot of an overnight culture of B. subtilis was added to 25 ml melted soft nutrient agar supplemented with 16 mM Ca(NO₃)₂. A negative control, a similar set of plates was prepared without calcium. Agar plugs were removed from the plates, creating wells into which 40 μl of the collected supernatant was added. Calcium-dependent antibiotic bioactivity was observed as a zone of inhibition after overnight incubation at 37°C.

**RESULTS**

The S. coelicolor genome encodes a protein with high similarity to B. subtilis RNases J1 and J2. A BLAST (25) search of the S. coelicolor genome, using RNases J1 and J2 as query sequences, revealed SCO5745 (SC9A10.09), which encodes a 561-amino-acid protein whose sequence is 39% identical and 60% similar to the B. subtilis RNase J1 protein and 38% identical and 60% similar to B. subtilis RNase J2. A PILEUP multiple-sequence alignment (26) is shown in Fig. 1. Analysis of the amino acid sequence showed conservation of the three major domains of RNase J necessary for RNase activity: the β-lactamase core (motifs 1 to 4), the β-CASP (elements A to C), and the C-terminal domain. These findings strongly suggest that SCO5745 is a member of the RNase J family.

To characterize the biochemical activity of SCO5745, the gene was cloned into pET19B and overexpressed in E. coli. The protein, with a decahistidine tag, was purified by immobilized metal ion chromatography. A homogeneous protein with an Mr of ca. 62,000 was obtained as judged by LDS-PAGE and Coomassie blue staining. Given the importance of the 5'-end phosphorylation state of substrates for RNase J activity, the enzyme preparation was...
assayed for pyrophosphatase contamination. Briefly, $[\alpha^{32}P]ATP$ was incubated with SCO5745 for 90 min. Reaction mixtures contained increasing amounts of cold ATP to facilitate enzyme activity, and the products were compared to ATP and CIP-treated ATP via their separation by thin-layer chromatography. SCO5745 digests produced no AMP, and thus there appears to be no contaminating pyrophosphatase in our preparations (see Fig. S1 in the supplemental material). In an additional attempt to detect phosphatase or pyrophosphatase contamination, we incubated SCO5745 with a chromogenic nonspecific substrate for phosphatases, $p$-nitrophenylphosphate ($pNPP$). The SCO5745 preparation was inactive against this substrate. Calf intestine alkaline phosphatase served as the positive control for the reaction and cleaved $pNPP$ efficiently (data not shown). Thus, our enzyme preparation does not appear to contain contaminating phosphatase or pyrophosphatase activities. One possibility that we have not been able to exclude definitively is contamination of the SCO5745 preparation with RNA pyrophosphohydrolase (RppH) from the *E. coli* strain used for overexpression. However, we note that we obtained results identical to those presented below (see Fig. 4 and 5) with an enzyme which had been subjected to additional purification steps (ion-exchange chromatography and gel filtration) for crystallization studies (data not shown). Taken together, our analyses suggest that SCO5745 itself, rather than contaminating enzymes, is responsible for the activities we observed in the studies reported here.

**The exonuclease activity of SCO5745 has 5′-to-3′ directionality.** To address the question of exonuclease directionality, we used the *thrS* RNA that is derived from the leader region of the threonyl-tRNA synthetase gene of *B. subtilis* (15, 16). A schematic structure of the *thrS* leader is shown in Fig. 2. Site 1 and site 2 are the mapped 5′ ends resulting from RNase J1 processing. Site 1 was originally thought to be produced by internal cleavage (4). However, processing is sensitive to the 5′ phosphorylation state and is produced via exonucleolytic activity (27). Site 2 is cleaved endonucleolytically by RNase J1 (4), by the *M. smegmatis* RNase J (5), and by *E. coli* RNase E (28). Curiously, the RNase J1/J2 heterotrimer does not cleave at site 2 but rather at a site immediately downstream of the terminator (10).

If SCO5745 functions as a 5′-to-3′ exonuclease, our expectations were that label would be released and accumulate more rapidly from a 5′-end-labeled substrate than from a 3′-end-labeled substrate. To examine the directionality of exonuclease digestion, therefore, we utilized 5′-end-labeled, monophosphorylated *thrS* and *thrS* labeled at the 3′ end with $[^{32}P]pCp$. As indicated in Materials and Methods, the two radioactive substrates were adjusted to the same specific activity using nonradioactive *thrS*. Digestion products were resolved by thin-layer chromatography and observed by autoradiography. *Figure 3A* shows that by 60 min, there was significant accumulation of both 5′- and 3′-end labels. *Figure 3B* shows the quantification of that accumulation by densitometry of the product spots. As expected, the rate of product accumulation was greater for the 5′-end-labeled substrate than for the 3′-end-labeled substrate. We conclude from this analysis that, like other RNases J, the exonucleolytic activity of SCO5745 proceeds 5′ to 3′.

**SCO5745 has both 5′-to-3′ exonucleolytic and endonucleolytic activities.** The endonucleolytic activity originally ascribed to RNases J1 and J2 (4) is now recognized as dependent upon high *in vitro* concentrations of enzyme (29, 30). Thus, we began the biochemical characterization of SCO5745 with an enzyme concentration curve. Since the exonuclease activity measured for other RNases J is strongly dependent upon the phosphorylation state of the 5′ end (4, 9, 11, 12), we used 5′-end-labeled monophosphorylated substrate. The results (Fig. 4) show that SCO5745 had robust exonuclease activity, as reflected by release of the monophosphate label at lower protein levels and decreased release of monophosphate at higher protein concentrations. We return to the latter observation below. In contrast, the endonucleolytic activity of SCO5745, reflected by the formation of 5′-end-labeled bands with mobilities between those of the full-length substrate and the monophosphate bands, was increased at higher concentrations of protein. Based upon these results, we chose to use an enzyme-to-substrate ratio of ca. 1.2 μg (19 pmol) SCO5745 per pmol of *thrS* in further experiments. This enzyme-to-substrate (E:S) ratio of 19:1 is close to that used by Mathy et al. (10).

**Activity of SCO5745 against 5′ monophosphorylated and 5′ triphosphorylated substrates.** As mentioned above, the exo- and

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**FIG 2** Schematic diagram of the *thrS* leader region. J1 site 1 and J1 site 2 indicate the *B. subtilis* internal cleavage sites as originally identified, with structure and numbering adapted from a report by Even et al. (4). Site 1 cleavage is now known to be produced by exonucleolytic activity.

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endonuclease activities of other characterized RNases J have been shown to depend on the phosphorylation state of the RNA substrate. It was therefore of considerable interest to determine whether 5'-end phosphorylation affected the activity of SCO5745.

**Figure 5.** Endonuclease activity of SCO5745 on mono- and triphosphorylated 5'-end-labeled thrS RNA. To obtain maximum resolution of the products of these assays, reaction mixtures were fractionated on a 12% sequencing gel, with two nucleoside phosphate standards, GMP and GTP. Monophosphorylated substrate was digested to GMP by SCO5745 in a time-dependent manner (Fig. 5, lanes 1 to 4). This substrate also produced internal cleavage products consisting of a few nucleotides proximal to the 5' end, as well as a major product created by cleavage presumably near site 2 of Fig. 2 (Fig. 5, arrows). This larger product was also produced from the triphosphorylated substrate (Fig. 5). Endonuclease activity proximal to the 5' end produced a ladder of small 5'-triphosphorylated products, 2 to 10 nucleotides in length. To our surprise, GTP, the expected product of exonuclease activity acting upon the triphosphorylated substrate, did not accumulate when that RNA was utilized in the reactions (Fig. 5, lanes 5 to 8). Thus, under our reaction conditions, SCO5745 is a bifunctional RNase. The predominant activity with a 5' monophosphorylated substrate is exonucleolytic, while the predominant activity with a 5' triphosphorylated substrate is endonucleolytic.

**SCO5745 is not essential, and disruption of the gene affects antibiotic production.** Model organisms that contain different sets of RNases have already revealed important alternative mechanisms of RNA metabolism (1, 31, 32). In *E. coli*, RNase J is absent and RNase E is the key enzyme for initiating mRNA degradation (31, 32). RNase E is absent in *B. subtilis*, but the organism contains a different endoribonuclease, RNase Y (33). As indicated above, RNase J was first identified in *B. subtilis* and for some years it was thought that both RNases J1 and Y were essential in that organism (33, 34). Recent studies have shown, however, that neither is essential in *B. subtilis* (35). In the actinomycete *M. smegmatis*, RNase E is essential while RNase J is not (5). *S. coelicolor* is known to contain RNase E, which is not essential (36), and the results reported here indicate that the organism also contains RNase J. It was of considerable interest, therefore, to determine whether SCO5745 was essential in *S. coelicolor*.

We replaced *sco5745* with a viomycin resistance cassette utilizing PCR-targeted mutagenesis as described by Gust et al. (21) to create the disruptive strain JSE2301. Positive disruptants were verified by PCR using primers 5745OutF1 and 5745OutR1 (Table 1) located ca. 100 bp upstream and downstream, respectively, from the *sco5745* open reading frame. Successful disruption of the gene predicted the amplification of a 1,670-bp product, while the parental allele should produce a 1,856-bp product. As shown in Fig. 6A, we observed products of the expected sizes using chromosomal DNAs from the parental and disrupted strains as the tem-
We also assessed antibiotic production in the complemented strain JSE2301/pJSE2255 and the vector control strain JSE2301/pIJ8600. Complementation with sco5745 restored the production of actinorhodin at day 3. We also note the failure of the JSE2301/pIJ8600 control to produce normal levels of antibiotic even by day 5. This strain was grown on three antibiotics, viomycin, apramycin, and thiostrepton, and unlike JSE2301/pJSE2255, it lacks a wild-type RNase J gene and so might not be expected to grow as well.

We also assessed the ability of JSE2301 to make calcium-dependent antibiotic, in comparison with that of control strains. CDA levels were measured after 3 days of growth and showed that production of this antibiotic was also dramatically reduced in JSE2301 (Fig. 6C). A similar rationale as that indicated above may apply to the CDA assay, in which the JSE2301/pIJ8600 control also did not make as much CDA as either the wild type or JSE2301/pJSE2255. Thus, RNase J joins RNase III (39–41) as an RNase that dramatically affects the production of antibiotics in S. coelicolor.

We did not observe any obvious effect of the SCO5745 mutation on the rate of growth of JSE2301 in solid or liquid media, nor did we observe any obvious deficiencies in the ability of the mutant to sporulate.

DISCUSSION

Two model pathways for RNA degradation have emerged based primarily upon the different combinations of enzymes present in E. coli and B. subtilis. As a first step in E. coli, 5′ triphosphorylated ends of primary transcripts are cleaved to the monophosphorylated state by the RNA pyrophosphohydrolase RppH (42). This step creates the preferred substrate for RNase E, an endonuclease that degrades RNA into short segments, thus creating new 5′ monophosphorylated ends. Since E. coli possesses no 5′-to-3′ exonucleases, further degradation proceeds via 3′-to-5′ exonucleases such as PNPase and RNase II (1, 43). In B. subtilis, degradation begins in a similar manner with cleavage by RppH (44). The endonuclease RNase Y also prefers substrates with 5′ monophosphorylated ends, and it too becomes active downstream of RppH (45). Exonucleolytic degradation occurs 5′ to 3′ via RNase J1/J2 that, like RNase E, prefers 5′ monophosphorylated substrates, and exonuclease degradation also occurs 3′ to 5′ via PNPase. In contrast, S. coelicolor, although possessing an RNase E homolog, does not contain RNase Y, and no evidence for the presence of RppH has yet been adduced.

We have shown here that SCO5745 functions as both a 5′-to-3′ exonuclease and an endonuclease. Furthermore, we show that endonuclease activity can efficiently degrade the 5′ triphosphorylated thrS transcript without the release of GTP. Taverniti et al. (5) have proposed a model in which RNase J acts as a sliding endonuclease. Since the 5′ triphosphate moiety cannot fit into the phosphate binding pocket of the RNase J active site (27), Taverniti et al. suggest that the RNA slides past the catalytic center and is then endonucleolytically cleaved at downstream nucleotides close to the 5′ end (5). Our results are consistent with this model, as shown by the formation of small, ladderlike, 2- to 10-nucleotide bands resolved on denaturing polyacrylamide gels (Fig. 5). Taverniti et al. (5) further observed the release of GTP by what they claim is endonucleolytic cleavage, as presumably the 5′ triphosphate suppressed exonucleolytic activity. They also report a clear bias for two exonuclease cleavage sites: the first at site 2 (Fig. 2) and the
FIG 6 (A) PCR analysis of the sco5745 region in S. coelicolor M145 and the disruptant strain JSE2301. Lane 1, size standards; lane 2, PCR product from S. coelicolor M145; lane 3, PCR product from strain JSE2301. (B) Comparison of antibiotic production in S. coelicolor M145 and sco5745 disruptant strains. Equivalent amounts of spores were streaked onto R2YE medium and incubated at 30°C to permit comparison of undecylprodigiosin (red) and actinorhodin (blue) antibiotic production. Plates streaked with strains bearing derivatives of pIJ8600 contained apramycin (50 μg ml⁻¹) and thiostrepton (50 μg ml⁻¹) to induce expression from the tipA promoter. All plates contained nalidixic acid (25 μg ml⁻¹) and nystatin (245 U ml⁻¹). Plates bearing JSE2301 and its derivatives also contained viomycin (30 μg ml⁻¹). (C) CDA assay. Zones of inhibition of B. subtilis BG267 appeared after overnight incubation at 37°C of the indicator strain, BG267, in the presence of 16 mM Ca(NO₃)₂. The center wells contained 40 μl of supernatant from S. coelicolor strains grown for 3 days on Oxoid nutrient agar. No inhibition was seen when calcium nitrate was omitted from the medium (data not shown).
other encompassing the first four nucleotides proximal to the 5’ end of thrS. We saw a very similar internal cleavage pattern (Fig. 5); however, there was no release of GTP. de la Sierra-Galley et al. (27) also reported internal cleavage at site 2 but no release of GTP.

Was our failure to observe the release of GTP due to the relatively high protein concentrations used in our study, thus favoring endonucleolytic activity and inhibiting exonuclease activity? Our E:S ratio was 19, while Taverniti et al. (5) used an E:S ratio of 0.1. Daou-Chabo and Condon (30), using an E:S ratio similar to ours, described exonuclease activity as nonspecifically cleaving all single-stranded regions. Thus, one might expect endonucleolytic cleavage to release GTP even at high protein concentrations. We observed no GTP release (Fig. 5). This raises at least two questions. Was the release of GTP reported by others through exonuclease or endonuclease activity? Is the mechanism by which exonucleolytic activity is inhibited by the 5’-triphosphate moiety the same mechanism that causes stimulation of endonucleolytic activity by high E:S ratios? The answer to these questions will require further experimentation. Studies to determine the crystal structure of SCO5745 are in progress and should shed light on the answers to these mechanistic questions.

Ultimately, the important question is what does SCO5745 do in vivo? It has been shown in several systems that RNases J are active in rRNA maturation (5, 7). Chloroplast RNase J functions as an RNA surveillance mechanism for antisense RNA (9). In B. subtilis, an RNase J null mutation combined with reduced expression of RNase J1 globally affected the expression of over 600 genes (46). We have shown here that S. coelicolor contains only a single copy of RNase J, which is not essential, and that knocking out sco5745 delays the onset of actinorhodin production while undecylenodipigiosin is overproduced and CDA production is greatly reduced. Thus, at the very least, it is clear that SCO5745 functions in the control of antibiotic production in S. coelicolor.

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