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# Species-Specific Cleavage by RNase E-Like Enzymes in 5S rRNA Maturation

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**Abstract** Previous work has identified a *Streptomyces coelicolor* gene, *rns*, encoding a 140 kDa protein (RNase ES) that exhibits the endoribonucleolytic cleavage specificity characteristic of RNase E and confers viability on and allows the propagation of *E. coli* cells lacking RNase E. Here, we identify a putative *S. coelicolor* 9S rRNA sequence and sites cleaved by RNase ES. The cleavage of the *S. coelicolor* 9S rRNA transcript by RNase ES resulted in a 5S rRNA precursor (p5S) that had four and two additional nucleotides at the 5' end and 3' ends of the mature 5S rRNA, respectively. However, despite the similarities between RNase E and RNase ES, these enzymes could accurately process 9S rRNA from just their own bacteria, indicating that these ancient enzymes and the rRNA segments that they attack appear to have co-evolved.

**Key words:** RNase E, RNase ES, 9S rRNA, 5S rRNA, p5S, RNA processing

Ribonuclease E (RNase E), an *Escherichia coli* endoribonuclease was initially discovered because of its ability to generate p5S RNA from nascent ribosomal 9S rRNA transcripts [6]. It has since been shown to have various processes of other catalytic and structural RNAs [7, 19, 20, 22, 27, 35], degrade multiple mRNAs [4, 33], and regulate the synthesis of certain plasmid DNAs [21].

RNase E contains three functionally distinct domains. The catalytic function of RNase E resides in the N-terminal half of the protein (amino acid residues 1 to 498), which also contains cleavage site specificity [24]. Meanwhile, the arginine-rich RNA-binding domain located between amino acids 580 and 700 has been found to enhance the cleavage of certain substrates [23, 26], and the proline-rich/acidic C-terminal third of the RNase E protein serves as a scaffold for the formation of a multi-component 'degradosome' complex that includes polynucleotide phosphorylase (PNPase), the RhlB RNA helicase, the ATP-generating enzyme enolase, the chaperon proteins DnaK and GroEL, polyphosphate kinase, and poly(A) polymerase [2, 25, 28–30, 34]. The existence of an RNA degradosome-like complex has been also recently found in *Streptomyces coelicolor* and *Rhodobacter capsulatus*, although PNPase, which is a major component of the *E. coli* degradosome, was not identified in the complex found in *Rhodobacter capsulatus* [10, 17].

Putative proteins containing evolutionarily conserved sequences resembling those present in the catalytic domains of *E. coli* RNase E and/or its paralog RNase G are encoded by the chromosomes of many bacterial species and a few eukaryotes [5, 17]. The eubacterial kingdom can be divided into two groups according to the type of ribonuclease that carries out the 5S rRNA maturation; one group carries RNase E and/or G, whereas the other carries RNase M5 that has been recently shown in *Bacillus subtilis* to cleave the 5S precursor in a double-stranded region to yield the mature 5S rRNA in one step [5].

Earlier work aimed at understanding the role of RNA decay in the control of gene expression in a widely studied streptomycetes, *S. coelicolor*, demonstrated that the *S. coelicolor* gene, named *rns*, encodes an endoribonuclease (RNase ES) that can functionally substitute for Rne in *E. coli*, attacks oligonucleotides and other substrates at or near known RNase E cleavage sites, and like *E. coli* RNase E, interacts with PNPase encoded by its host species to form an RNA degradosome-like complex [17]. Notwithstanding these similarities, we found that RNase E and RNase ES were able to process 9S rRNA into p5S from only their own bacteria, indicating that these ancient enzymes had congruently evolved with species-specific rRNA processing mechanisms. Accordingly, this study investigated a possible physiological role of RNase ES in *S. coelicolor*, which is a morphologically, developmentally, and biochemically complex soil bacteria that can synthesize a variety of biologically important secondary metabolites, including a large portion of antibiotics in medical and veterinary uses [3, 8, 12, 14, 31, 32, 36].

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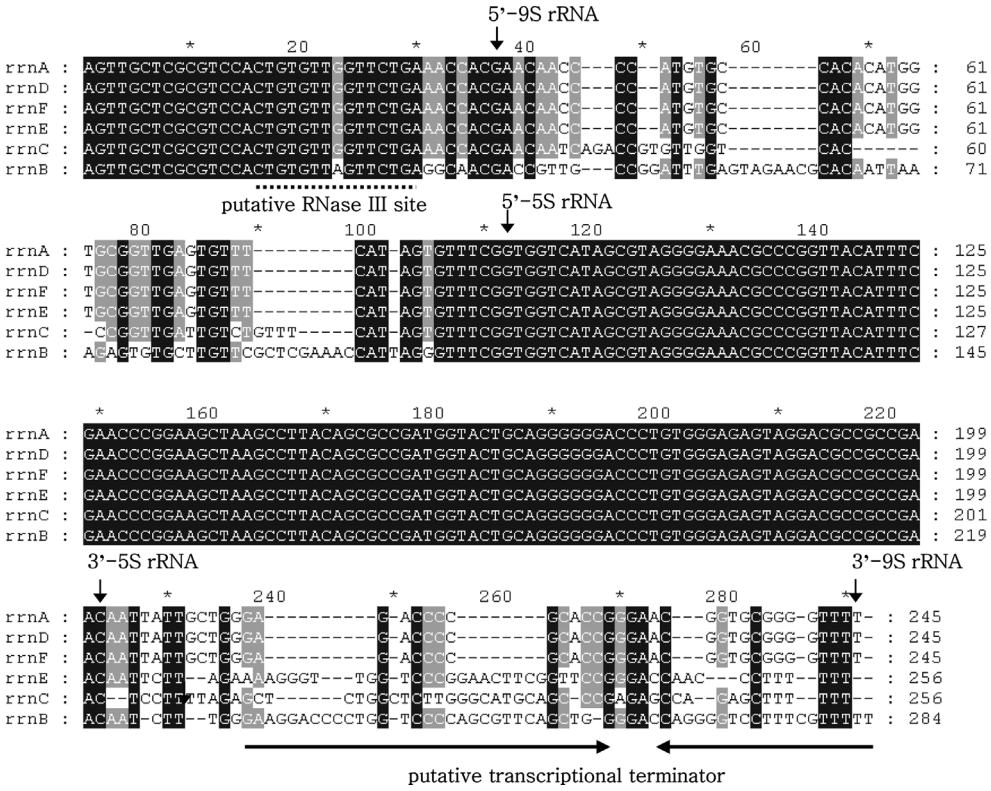


**Fig. 1.** Alignment of sequences from the rDNA region encompassing *S. coelicolor* 5S rRNA.
The putative RNase III site and transcriptional terminator are underlined with a broken line and converging arrows, respectively. The sequences were aligned using the Clustal W program.

## MATERIALS AND METHODS

### Northern Blot Analysis

The isolation of total RNA from *S. coelicolor* and the Northern blot analysis were both performed as previously described in [11] and [16], respectively.

### Protein Work

The ribonucleases were all purified using a HisBind purification kit (Novagen), as previously described [17].

### Synthesis of RNA and *In Vitro* Cleavage Assay

The synthesis of $^{32}$P-labeled *E. coli* 9S rRNA and the RNase E cleavage assay were performed as previously described [16]. The *S. coelicolor* 9S rRNA was synthesized from PCR-amplified DNA containing the SP6 promoter, 5S rRNA sequence, and an additional 53 and 43 nucleotides at the 5' and 3' end, respectively, using a MEGAscript SP6 kit (Ambion, Austin, TX, U.S.A.). The primers used to synthesize the PCR DNA from the *S. coelicolor* chromosomal DNA were 5'Sc-9S (5'ATGGATCCATTTA-GGTGACACTATA**GAAACCACGAACAACCCCATG**, SP6 promoter sequence is underlined) and 3'Sc-9S (5'**AAA-ACCCCGCACCGTTCCCGGT**). These PCR primers were designed to anneal to the same sequences (complementary sequences are in bold type in the primer sequences) found in the ribosomal RNA operons, *rrnA*, *rrnD*, and *rrnF*.

### Primer Extension

The cDNA was synthesized using avian myeloblastosis virus (AMV) reverse transcriptase. The primer 5S rRNA-

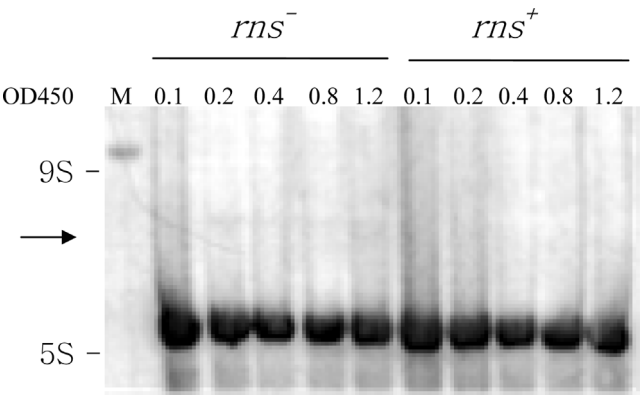


**Fig. 2.** Northern blot analysis of 5S rRNA precursors.
The 5S rRNA precursors were identified by probing with 5S rRNA (5'AGGCTTAGCTTCCGGGTTCG). To prepare the total RNA from the *rns*-deleted cells and their parental strain, *S. coelicolor* A3(2), the culture was grown in a YEME medium and harvested at $OD_{450}$=0.1, 0.2, 0.4, 0.8, and 1.2. Lane M contained 10 ng of the *S. coelicolor* 9S transcript used in Fig. 3C.

43L (5'AGGCTTAGCTTCCGGGTTCG), which was complementary to the 5S rRNA transcript, was labeled with [γ-$^{32}$P]ATP at the 5'-end using T4 polynucleotide kinase and purified from 15% acrylamide containing 8 M urea. The reaction was performed as previously described [15], except that sequencing the reactions contained all four dNTPs and each ddNTP.

## RESULTS AND DISCUSSION

### Identification of 9S Ribosomal RNA Transcripts in *S. coelicolor*

RNase E was initially discovered by its ability to process 9S rRNA in *E. coli* cells, and the shift of *rne ts* bacteria to a non-permissive temperature lead to the *in vivo* accumulation of precursors of 5S rRNA [6]. Thus the discovery of a functionally similar, structurally shuffled ortholog of *E. coli* RNase E in *S. coelicolor* [17] prompted an examination of the involvement of RNase ES in the processing of 9S rRNA in *S. coelicolor*. Since the complete genomic DNA sequence for *S. coelicolor* recently became available [1], this enabled the identification of putative 9S rRNA coding regions by searching for a putative 9S rRNA sequence based on comparing the rDNA regions of *S. coelicolor* with those of *E. coli* and another *Streptomyces* species, *Streptomyces griseus*. The putative 9S rRNA sequences identified were found to be preceded by a segment for an RNase III processing site (Fig. 1). The *S. coelicolor* genome contains six rRNA operons (*rrnA-F*) and three of

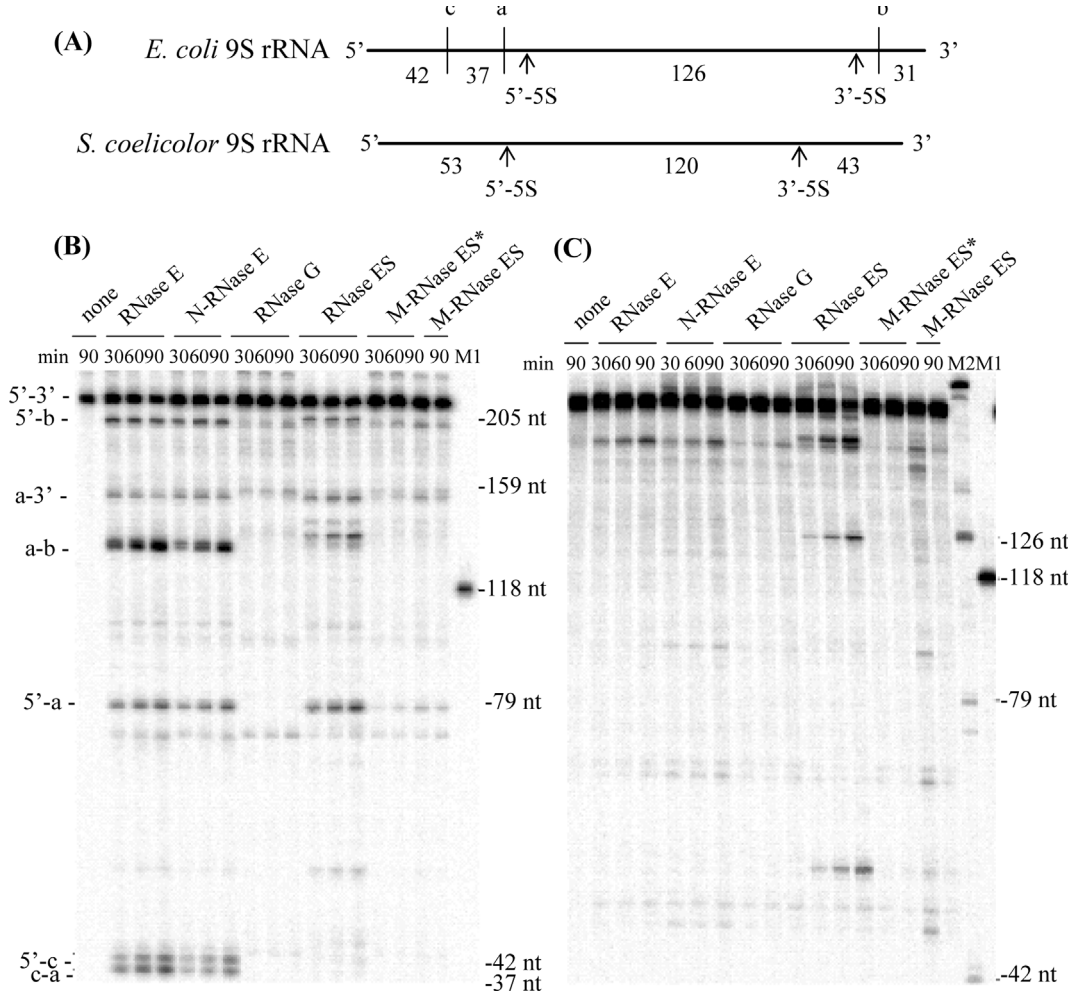


**Fig. 3.** Cleavage of 9S rRNA. (A) *In vitro* synthesized *E. coli* and *S. coelicolor* 9S rRNA transcript.
The RNase E cleavage sites are indicated as a, b, c, and the 5' and 3' termini of the mature 5S rRNA are indicated as 5'-5S and 3'-5S, respectively. *E. coli* (B) and *S. coelicolor* (C) 9S rRNA cleavage *in vitro*. One pmol of the *in vitro* synthesized 9S rRNA transcript internally labeled with $^{32}$P-UTP was incubated with no protein, 100 ng (RNase E and RNase ES), or 200 ng (RNase G, N-RNase E, and M-RNase ES) of purified protein in 40 μl of a 1× cleavage buffer at 37°C. Ten μl of each sample was removed at each time point indicated and mixed with an equal volume of a formamide-containing loading buffer and loaded onto a 9% polyacrylamide gel containing 8 M urea. Lane M1 contained one pmol of the *in vitro* synthesized GGG-RNAI transcript internally labeled with $^{32}$P-UTP, and lane M2 contained one pmol of the *in vitro* synthesized *E. coli* 9S rRNA transcript internally labeled with $^{32}$P-UTP that had been cleaved by RNase E.

them (*rrnA*, *rrnB*, and *rrnF*) were found to contain an identical sequence flanking a putative 9S rRNA. The arrangement of the rRNAs and putative RNase III sites in the *S. coelicolor* rDNA was very similar to that found in *E. coli* rDNA, and another *Streptomyces* species, *Streptomyces griseus* [13].

To test the involvement of RNase ES in the maturation of 5S rRNA, the total RNA was prepared from *rns*-deleted cells and their parental strain, *S. coelicolor* A3(2), and a Northern blot analysis was performed. As shown in Fig. 2, a faint yet distinct band larger than 5S rRNA was hybridized to a 5S rRNA-specific probe in the lanes loaded with RNA isolated from only the *rns*-deleted cells, indicating the existence of a 5S rRNA precursor and the involvement of RNase ES in 5S rRNA maturation.

### *In Vitro* Processing of 9S rRNA by RNase ES

Next, the ability of RNase ES to cleave the 9S rRNA transcripts derived from *E. coli* and *S. coelicolor* was tested. In contrast to other *E. coli* RNase E substrates tested previously [17], RNase ES did not generate a cleavage product of the same size as p5S, although it did cleave the *E. coli* 9S rRNA transcript (Fig. 3A). Instead, RNase ES generated a larger cleavage product than the size of p5S and appeared to cleave at site 'a' and at site 3' to site 'b', whereas M-RNase ES seemed to cleave only at site 'a' (Figs. 3A and 3B) when comparing the size of the cleavage products. This result then prompted a further test to determine the ability of the enzymes to cleave the *S. coelicolor* 9S rRNA transcript. This transcript was derived from the common sequence present in the *rrnA*, *rrnD*, and *rrnF* operons containing a 5S rRNA sequence plus additional 53 and 43 nucleotides at the 5' and 3' ends, respectively (Fig. 1). Interestingly, only RNase ES was able to generate a cleavage product similar to the size of the *E. coli* p5S, which is 126 nucleotides long (Fig. 3B). Therefore, these results indicate that RNase E and RNase ES can generate a cleavage product of p5S from just their own bacterial 9S rRNA, whereas RNase ES possesses the catalytic properties and cleavage activities of RNase E on most RNase E substrates (RNAI, BR13, and pM1 RNA) and complements an *E. coli* null mutation in *rne* [17].

The cleavage sites of the *S. coelicolor* 9S rRNA transcript were further identified by synthesizing the cDNA with the RNA extracted from the cleavage reaction as the template (Fig. 4). A primer extension analysis revealed that the RNase ES cleavage at the site proximal to the 5'-end of the transcript was four nucleotides away from the 5'-end of the 5S rRNA and resulted in a 5' extension containing four additional nucleotides indicating the presence of a further processing by unknown mechanisms, as previously reported in the maturation of *E. coli* 5S rRNA [6]. The RNase ES cleavage site proximal to the 3'-end of the transcript was deduced from the size of the cleavage product (126 nt),

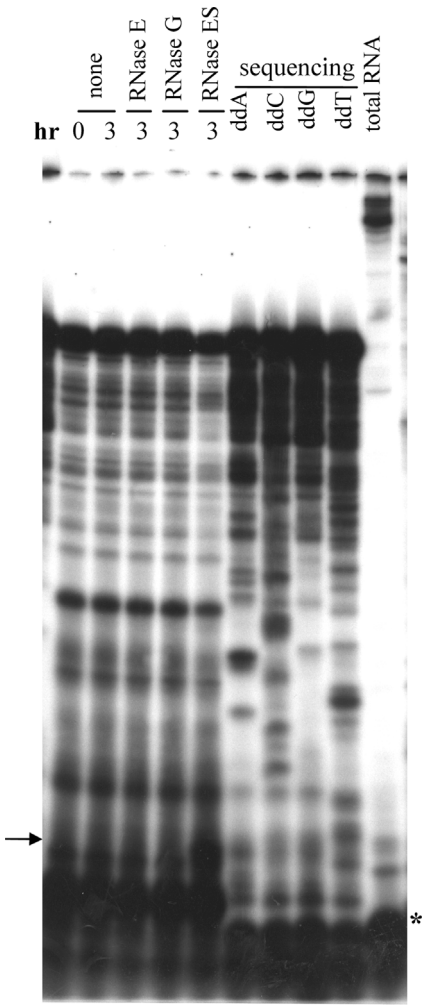


**Fig. 4.** Identification of RNase ES cleavage site proximal to the 5'-end of 5S rRNA.

The RNA was isolated from the same cleavage reaction described in Fig. 3C and the cDNA was synthesized using AMV reverse transcriptase and a 5'-$^{32}$P-end labeled oligo complementary to 5S rRNA (5'AGGCTTAGCT-TCCGGGTTCG). For the sequencing ladders, dideoxyribonucleotides were added additionally to the reaction with the uncleaved 9S rRNA transcript as a template. In the last lane, the cDNA was synthesized with the total RNA isolated from the *rns*-deleted *S. coelicolor* A3(2) as a template. The cDNA synthesized from the 9S rRNA transcript cleaved by RNase ES is indicated by an arrow. In the last lane, the cDNA synthesized from the 5S rRNA in the total RNA sample is indicated by an asterisk.

which resulted in an extension of two additional nucleotides to the 3'-end of the 5S rRNA. In *E. coli*, the 3' extension is subsequently trimmed by an exoribonuclease, RNase T [18]. Interestingly, a BLAST search for RNase T homologs revealed no obvious sequence similar to RNase T in the *S. coelicolor* genome. In fact, among 116 organisms containing a genomic sequence(s) similar to *E. coli* and/or *S. coelicolor* RNase E/G homologs, only gamma proteobacterial species (46 out of 50) showed an obvious RNase T homolog, implying that other organisms possess

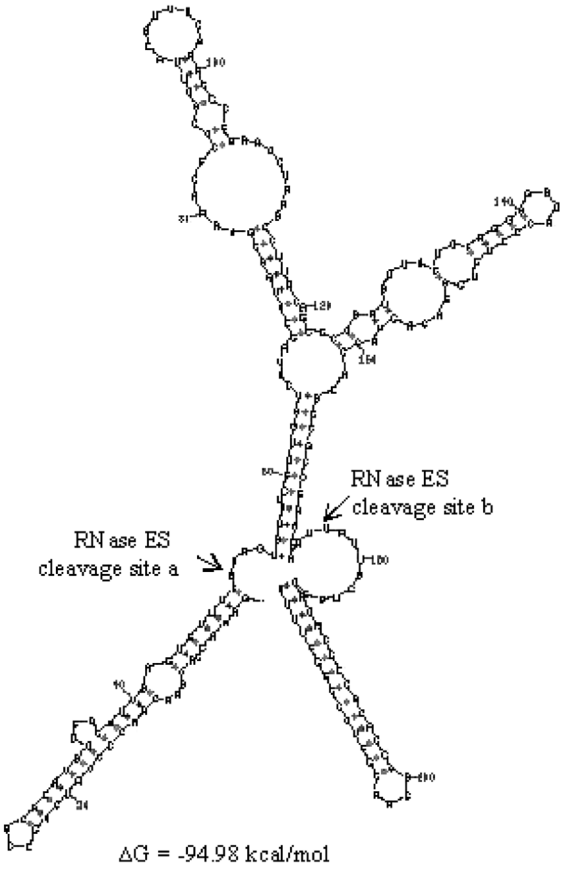


**Fig. 5.** Secondary structure of putative *S. coelicolor* 9S rRNA. The secondary structure for the 9S rRNA transcript used in Fig. 3C was predicted using the Mfold program [9]. The structure shown had the lowest free energy, and five predicted structures selected for their low free energy showed single-stranded regions of RNase ES cleavage sites. The RNase ES cleavage sites 'a' and 'b' were deduced from Fig. 4 and Fig. 3, respectively.

different mechanisms for the final processing reaction to produce the mature 3'-end of 5S rRNA.

A cDNA synthesis using a total RNA sample from *rns*-deleted *S. coelicolor* cells produced three cDNA species with size matching or one to two nucleotides shorter than the cDNA synthesized with the 9S rRNA where the 5'-segment was cleaved by RNase ES. When comparing the intensity of these bands, a further processing at the 5'-end of p5S appeared to occur by a sequential cleavage from the 5'-end. The predicted secondary structures of the putative *S. coelicolor* 9S rRNA showed that the identified cleavage sites were in single-stranded regions, further supporting that the sites are RNase ES processing sites for the maturation of 5S rRNA in *S. coelicolor* (Fig. 5).

It has already been shown that the classification of RNase E/G-like proteins according to the relative position of possible catalytic versus possible scaffold regions in these proteins provides a phylogenetic tree that is in good agreement with a phylogenetic classification made on the basis of small-subunit ribosomal RNA [17]. Consequently, given the present observation that RNase E and RNase ES can process 9S rRNA into p5S from just their own bacteria, despite the ability of RNase ES to confer viability on *E. coli* cells deleted for *rne*, the RNase E/G ribonucleases and the rRNA segments appear to have congruently evolved.

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