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Heterogeneous rRNA Molecules Encoded by *Streptomyces coelicolor* M145 Genome are All Expressed and Assembled into Ribosomes

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Abstract The *Streptomyces coelicolor* M145 genome harbors six copies of divergent rRNA operons that differ at ~0.2% and ~0.6% of the nucleotide positions in small subunit (SSU) and large subunit (LSU) rRNA genes, respectively. When these rRNA genes are expressed, a single cell may harbor three different kinds of SSU rRNA and five kinds of LSU rRNA. Primer extension analyses revealed that all of the heterogeneous rRNA molecules are expressed and assembled into ribosomes. This finding and the maintenance of the intragenomic variability of rRNA operons imply the existence of functional divergence of rRNA species in this developmentally complex microorganism.

Keywords: *Streptomyces coelicolor*, rRNA genes, SSU rRNA, LSU rRNA, heterogeneous rRNA

Like most other bacteria, *Streptomyces* species contain multiple copies of ribosomal RNA operons and rRNA genes that are closely linked and organized in the order of 16S-23S-5S [1, 4, 7, 9, 10, 15, 17, 18]. It is generally assumed that all rRNA sequences in a single organism are identical, and this has been the basis for using rRNA genes for identification and classification of microorganisms for several decades [14]. During evolution, each organism came to have a unique rRNA species that serves best its physiological needs, although much of the structure and function of rRNA is evolutionarily conserved [3, 8]. However, sequence analysis of the *S. coelicolor* genome revealed that it harbors six copies of divergent rRNA operons that may constitute three kinds of small subunit (SSU) rRNA and five kinds of large subunit (LSU) rRNA in a single cell (Fig. 1). It encodes only one kind of 5S rRNA, as previously shown [12]. The most variable region was present in the region between nucleotide positions 632 and 652 in LSU rRNA that corresponds to the helix 533 in *Escherichia coli* LSU rRNA. In *E. coli*, this variable region is part of the stem composed of two base pairs between positions 543–550 and 544–549 adjacent to a tetra loop, whereas, in *S. coelicolor*, the corresponding stem is extended to 8–9 base pairs [3].

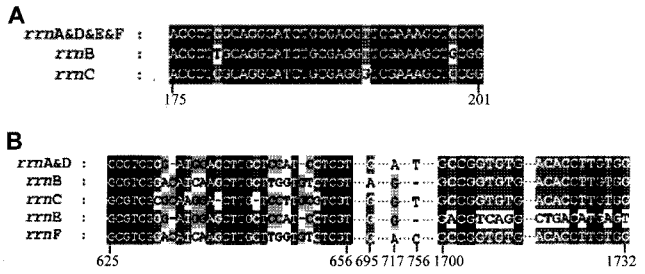

Fig. 1. Nucleotide distributions in the variable regions of *S. coelicolor* rRNA. Nucleotide positions correspond to rRNA sequences from the *rrn*F operon.
A. SSU rRNA, **B.** LSU rRNA.

Intragenomic heterogeneous rRNA genes were also found in another completely sequenced genome of *Streptomyces* species, *Streptomyces avermitilis* MA-4680, that harbors six copies of divergent rRNA operons [6]. These findings indicate that the heterogeneity of rRNA genes is a common phenomenon in *Streptomyces* species. However, it has not been shown whether the heterogeneous rRNA molecules are expressed and assembled into ribosomes in *Streptomyces* species.

This prompted us to set out experiments to detect each species of rRNA molecules in total RNA and rRNA isolated from ribosomes, to investigate whether heterogeneous rRNA species encoded by the *S. coelicolor* genome are expressed and assembled into ribosomes. Total RNA and

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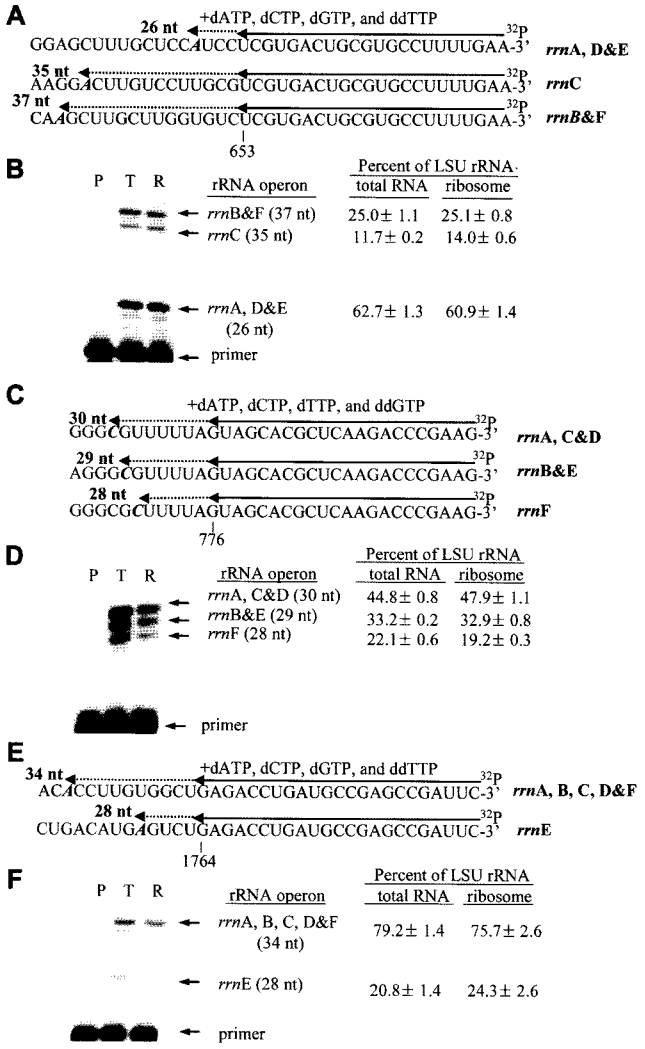

Fig. 2. Existence of heterogeneous LSU rRNA species in *S. coelicolor.*

Schematic representations of the modified primer extension method are shown (**A**, **C**, and **E**). The cDNA products synthesized from specific LSU rRNA templates were resolved in a 10% sequencing gel (**B**, **D**, and **F**). Relative abundance of each group of rRNA species in total RNA (lane T) or in ribosomes (lane R) was measured by OptiQuant image analysis software. ^{32}P-end-labeled primer complementary to the 3' region of the variable region was extended using AMV reverse transcriptase. The extension mixture contained three kinds of dNTPs and one kind of ddNTP, as indicated. In lane P, samples from the extension reaction carried out without RNA was loaded. The nucleotide position in rRNA to which the 3'-end of each primer binds is indicated. Total RNA was prepared using an RNeasy Kit (Qiagen) according to the manufacturer's recommendations. Crude ribosomes were isolated by the method of Powers and Noller [11]. Ribosomal RNA was purified from the crude ribosomes using phenol extraction and ethanol precipitation.

rRNA from cells grown in chemically defined (CD) medium [13] were isolated, and the existence of heterogeneous rRNA species with sequence variations at specific positions in RNA samples were quantitatively detected using a modified primer extension method [8]. Fig. 2A describes the method that utilizes extension of the primer annealed to the 3' region of the variable region of LSU rRNA (positions between 631 and 652) in the presence of three kinds of dNTPs (dATP, dCTP, and dGTP) and one kind of ddNTP (ddTTP). Three groups of LSU rRNA species can be detected using the primer, as shown in Fig. 2A. The relative proportions of rRNA in the sample with specific mutations can be identified from the relative intensities of the three differently terminated cDNA bands (Fig. 2B). The results of the primer extension showed that LSU rRNA molecules containing the same variable sequences (nucleotide positions between 631 and 652) that are derived from three operons (*rrn*A, D, and E) constituted approximately 60% of total LSU rRNA, whereas LSU rRNA molecules from *rrn*B and F were approximately 25% of total LSU rRNA and those from the *rrn*C operon occupied about 15%. Distributions of hetergeneous rRNA species in total RNA were similar with those in ribosomes, indicating that they were equally well assembled into ribosomes.

Since three groups of heterogeneous LSU rRNA molecules were expressed and assembled into ribosomes, we further tested the existence of each LSU rRNA species in the cell utilizing a similar method described above with primers that bind to other variable regions of LSU rRNA (Figs. 2C and 2E). The results showed that all LSU rRNA species

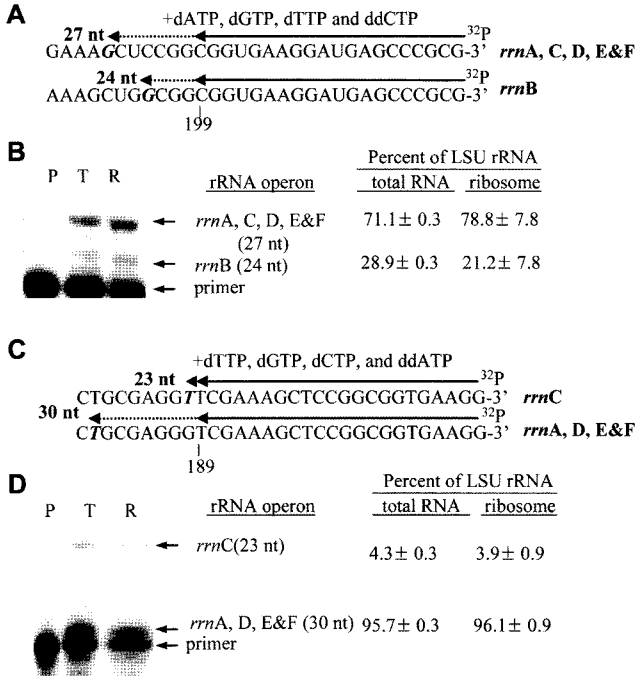

Fig. 3. Existence of heterogeneous SSU rRNA species in *S. coelicolor.*

Schematic representations of modified primer extension method are shown (**A** and **C**). The modified primer extension method described in the Fig. 2 legend was used to detect heterogeneous SSU rRNA molecules and measure the relative abundance of each SSU rRNA species in total RNA (lane T) or in ribosomes (lane R). In lane P, samples from the extension reaction carried out without RNA was loaded.

were expressed in the cells and assembled into ribosomes (Figs. 2B, 2D, and 2F). LSU rRNA molecules containing the same sequences transcribed from two operons (*rrn*A and D) constituted approximately 40% of total LSU rRNA, whereas the distribution of LSU rRNA molecules from *rrn*B, C, E, or F was approximately 10%–20% of total LSU rRNA. We also tested the existence of heterogeneous SSU rRNA molecules in the cell using similar methods described in Fig. 2. The results showed that SSU rRNA molecules containing the same sequences transcribed from the *rrn*A, D, E, and F operons occupied about 70% of the total SSU rRNA, respectively, whereas about 30% of SSU rRNAs were from the rest of the rRNA operons (Fig. 3). Once again, the results showed that all three different kinds of SSU rRNA species were expressed in the cells and equally well assembled into ribosomes. There was 7% to 8% difference in the distribution of SSU rRNA and LSU rRNA from *rrn*C in total rRNA, and this may be due to a lower resolution of cDNA bands in the gel shown in Fig. 3D.

Our results showed that all of the heterogeneous rRNA molecules are expressed and assembled into ribosomes in the *S. coelicolor* cell. The maintenance of the intragenomic variability of rRNA operons in the *S. coelicolor* genome by evolutionary pressure implies the existence of functional divergence of rRNA species. Such a relationship between intragenomic rRNA divergence and functionality has been observed in the apicomplexan *Plasmodium berghei*, where two types of SSU rRNA were differentially expressed depending on the different stages of the life cycle of this organism [5]. This phenomenon has also been indicated in extremely halophilic archaea, whose selective advantage could be gained from differential expression of divergent rRNA operons, depending on the salt concentration in the environment [2]. A specific function of heterogeneous rRNA has not been identified; however, it is tempting to hypothesize that heterogeneous rRNA may play a role in the control of gene expression by preferentially translating specific mRNA species depending upon certain physiological needs.

The maintenance of high intragenomic heterogeneity of rRNA genes in the *S. coelicolor* genome, and the expression and assembly of heterogeneous rRNA molecules into ribosomes observed here, suggest the existence of an unidentified regulatory circuit controlled by specific rRNA molecules to modulate gene expression at the translational level in this developmentally complex microorganism. We are currently analyzing expression profiles of LSU rRNA species during the different stages of morphological development as well as under specific nutritional conditions of the cells, and constructing strains deleted for each of the rRNA operons to identify the physiological role of heterogeneous rRNA molecules.

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