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Ribosomal RNA Operons in *Streptomyces rimosus*: Sequence of the *rrnF* and Comparative Analysis of *rrn* Promoter Regions

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Summary

One of the six *Streptomyces rimosus* ribosomal RNA operons, *rrnF*, was completely sequenced. rRNA genes, arranged in the order 16S-23S-5S, encode 1529, 3121 and 120 nucleotides long rRNAs, respectively. tRNA genes were not found in the 16S-23S spacer region, or at the 3' end of the operon. Only one putative promoter of the *rrnF* operon (P4) was identified by sequence similarity. Open reading frames, located on both sides of the *rrnF*, are conserved at the same positions in some other *Streptomyces* rRNA operons.

According to the conserved *Eco*RI sites, two more types of promoter regions exist in the five remaining *S. rimosus* rRNA operons. Three operons have type I promoter region and a sequenced representative contained four putative promoters (P1-P4). Type II promoter region, present in two operons, is most likely deletion derivative of the type I and misses P2 and P3 promoters. Only the most proximal promoter, P4, and downstream DNA sequences are highly conserved in all three analysed promoter regions of *S. rimosus* rRNA operons.

Key words: rRNA operon, multiple promoters, streptomycetes, X62884

Introduction

Like in most other bacteria, ribosomal RNA genes in *Streptomyces* are closely linked, separated by short intergenic sequences and organised in the order 16S--23S-5S. So far tRNA genes were not found in association with *Streptomyces* rRNA operons. The number of rRNA operons varies in different species. Four operons are present in *S. ambofaciens (1)*, six in *S. coelicolor (2)*, *S. lividans (3)*, *S. rimosus (4)*, *S. griseus (5)* and *S. nodosus (6)* and seven operons were so far found only in *S. venezuelae (7)*. For many *Streptomyces* species only partial sequences of rRNA operons or genes (mostly 16S rRNA genes) are known. Complete sequences were reported only for *rrnB* from *S. lividans (8)*, *rrnD* from *S. ambofaciens (1)*, *rrnE* from *S. griseus (5)* and for four (out of six) rRNA operons from *S. nodosus (6)*. 23S RNA genes are very long in *Streptomyces* (over 3100 bp), as a consequence of specific insertions in two variable regions. Homologies between rRNA genes from different streptomycetes are at least 90 % or higher. Ribosomal RNA synthesis was studied from *S. coelicolor rrnD (9)* and *rrnA* operon *(10)* and from three *S. lividans* operons *(11)*. Up to four promoters regulate the expression of rRNA operons in *Streptomyces*. Promoters differ in sequence, especially at –35 region. Multiple promoters are not equally active under different growth conditions *(9–11)*.

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This paper reports on the complete sequence of *rrnF* operon from *S. rimosus* and sequences of two aditional promoter regions of rRNA operons that were identified in *S. rimosus*.

Materials and Methods

Cloning of the *rrnF* operon from *S. rimosus* R6-554 and identification of *Eco*RI fragments encoding immediate upstream regions of five other rRNA operons were described earlier *(4)*. *S. rimosus Eco*RI fragments of interest were cloned in pBluescribe M13 and positives, encoding promoter regions of rRNA operons, were identified after hybridisation with a radiolabeled 5' end of the 16S rRNA gene. All DNA manipulations were performed by routine methods *(12)*. DNA sequences were determined from both ends using universal and several specific primers. Sequences were stored, compared and analysed using PC/GENE 14.0 computer programs from IntelliGenetics (Mountain View, USA). Homology searches were performed *via* the Internet server at NCBI (NIH, Bethesda, USA) using BLAST program.

Results and Discussion

The rrnF operon of S. rimosus

The *rrnF* operon from *S. rimosus* is located on 8.8 kb long *Bam*HI fragment *(4)*. 3' end of the operon, including 5S rRNA gene, was sequenced previously *(4)*. We extended the analysis and determined the sequence of the entire operon, including 689 bp upstream from the 5' end of 16S rRNA gene and 238 bp downstream from the 3' end of 5S RNA gene (Acc No. X62884). Shematic description of *rrnF* operon is shown in Fig. 1. As was expected, three rRNA genes are arranged in the order 16S-23S-5S and encode 1529, 3121 and 120 nt long RNAs, respectively. Homology with rRNA genes from other streptomycetes is in the range of 95 % for all three genes. Intergenic regions between 16S-23S rRNA genes (298 bp) and 23S-5S rRNA genes (72 bp) do not encode tRNA genes and show considerable homology (at least 70 %) with known intergenic regions of *Streptomyces* rRNA operons. Two inverted repeats are located at the 3' end of the operon and most probably represent *rho* independent transcription termination signals. Start of an ORF 172 bp from the end of 5S RNA gene (first 22 aa) was identified by sequence similarity (86 %) with putative CDP-diacylglycerol-glycerol-3-phosphate-3-phosphatidyl transferase from *S. coelicolor* (AL353861). In *S. coelicolor*, this ORF starts 70 bp downstream from the end of *rrnC* operon. Second ORF starts 593 bp upstream from the beginning of the 16S rRNA gene, runs in the opposite direction, and shows 96 % identity in the first 32 identified amino acids with putative PTS system sugar phosphotransferase component IIA from *S. coelicolor*, located at about the same position in *rrnC* operon. This ORF is also found 713 and 570 bp upstream from the 16S rRNA genes in *S. griseus rrnC* (AB030569) and *S. lividans rrnX* operon *(11)*, respectively.

Only one putative promoter (P4) was identified by sequence similarity in a DNA region upstream from the start of 16S rRNA gene (Fig. 2). The –10 box of P4 promoter reads TAGAGT (–275 to –269). The same –10 box was identified in numerous promoters of rRNA operons from streptomycetes (Table 1), while –35 regions vary considerably and often contain G and A rich DNA. High homology with upstream regions of other *Streptomyces* rRNA operons exists only in the last 280 bp. Only few nucleotides in front of the P4 promoter (–10 box) the homology is lost and the region between –280 and the conserved ORF seems to be specific only for *rrnF*. Homology with *rrnC* operons from *S. griseus* and *S. coelicolor* and with *rrnX* from *S. lividans* is regained again only 15–20 bp in front of the upstream ORF. Downstream from the P4 promoter is located a 21 bp long, conserved sequence (Fig. 2), found in front of all *Streptomyces* rRNA operons, that was defined as rRNA processing site *(1,13)*.

Promoter regions of other S. rimosus rRNA operons

By Southern hybridization analysis, we have previously identified only three differently migrating *Eco*RI fragments, which encode promoter regions of six *S.*

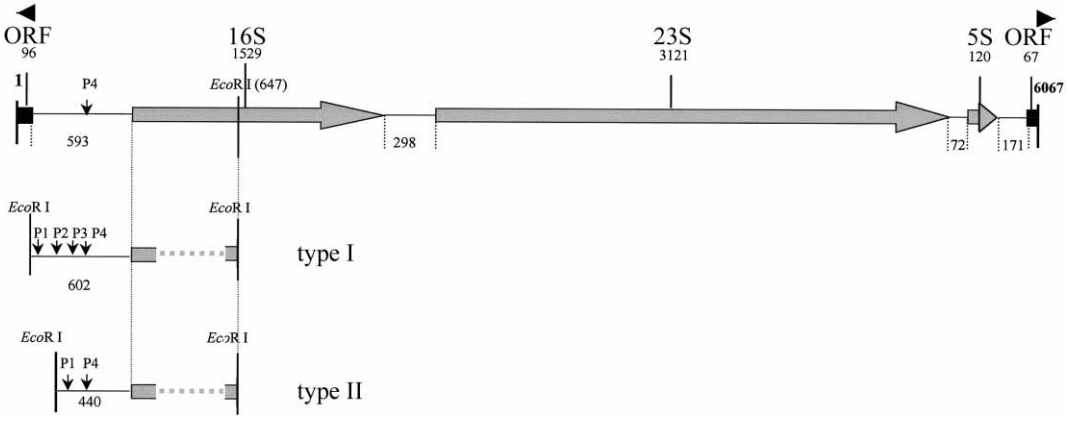

Fig. 1. Schematic description of the DNA fragment encoding *rrnF* operon from *S. rimosus* (top) (Acc No. X62884); type I and type II promoter regions present in other *S. rimosus* rRNA operons are shown below. Unsequenced parts of 16S rRNA genes are shown by dotted lines. Potential promoters (P1-P4), conserved *Eco*RI sites, coding regions of rRNA genes (16S, 23S and 5S), two putative open reading frames (ORFs) and intergenic regions are indicated. Corresponding lengths are given in base pairs

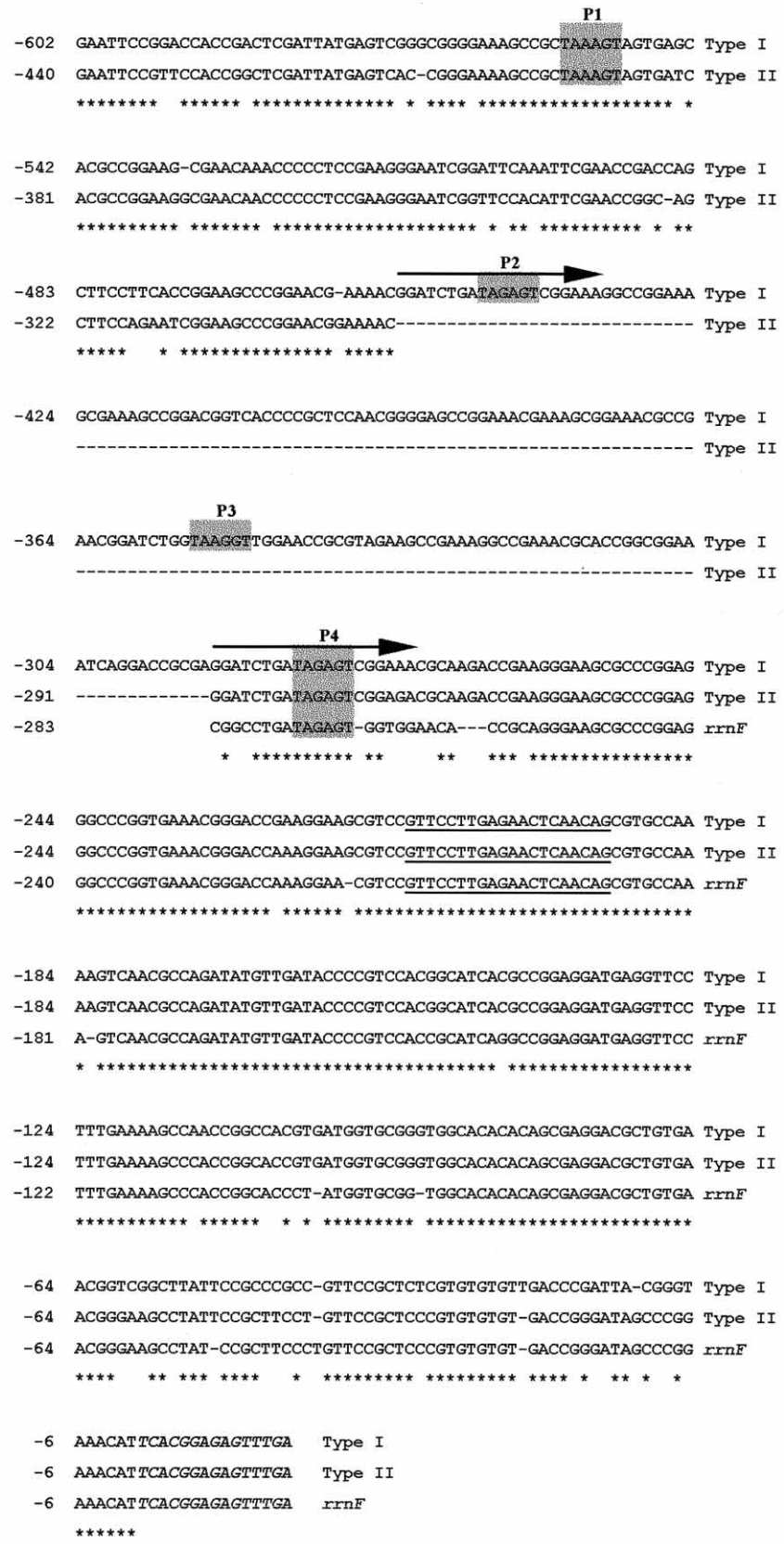

Fig. 2. Sequence alignment of type I, type II and *rrn*F promoter regions; type I and II DNA sequences are shown upstream from the beginning of the 16S rRNA coding region up to *Eco*RI site. From the *rrnF*, only the upstream region with significant homology with two other sequences was included in the alignment. Asterisks denote nucleotides that are identical and dashes indicate regions of deletion. Positions of potential promoters (P1-P4) are shadowed. A 20 bp long direct repeat in type I DNA is indicated by horizontal arrows. A putative rRNA processing site is underlined. The beginning of the 16S rRNA gene is shown in italics

Table 1. List of experimentally identified or proposed promoters of *Streptomyces* rRNA operons with –10 boxes identical to putative P1-P4 promoters from *S. rimosus*

S. rimosus –10 box	Species	rRNA operon	Promoter no.	Ref.
P1 (TAAAGT)	*S. griseus*	E	3	5
	S. ambofaciens	D	3	1
P2 and P4 (TAGAGT)	*S. lividans*	A	1	11
	S. lividans	A	3	11
	S. lividans	A	4	11
	S. lividans	F	2	11
	S. lividans	F	3	11
	S. lividans	F	4	11
	S. coelicolor	A	3	10
	S. coelicolor	A	4	10
	S. ambofaciens	D	4	1
	S. griseus	E	4	5
	S. nodosus	B	4	6
	S. nodosus	D	4	6
	S. nodosus	E	4	6
	S. nodosus	F	4	6
P3 (TAAGGT)	*S. lividans*	A	2	11
	S. coelicolor	A	1	10
	S. coelicolor	A	2	10
	S. nodosus	B	3	6

rimosus rRNA operons *(4)*. In this experiment 5' end of the 16S rRNA gene was used as a hybridization probe. The conserved *Eco*RI site starts at position 647 in all six *S. rimosus* 16S rRNA genes. The largest hybridising *Eco*RI fragment (over 20 kb) encodes promoter region of the *rrnF* operon, with the nearest upstream *Eco*RI site located over 20 kb from the 16S rRNA gene. Two other hybridizing *Eco*RI fragments were short in size (~1250 bp and ~1100 bp) and from the intensity of the signal they appeared to be triplet and doublet, respectively. Short *Eco*RI fragments were cloned and one representative of each size type was sequenced. In the type I promoter region (triplet), *Eco*RI site is located 602 bp upstream from the start of 16S rRNA gene and four putative promoters (P1-P4) were found in the DNA region between the *Eco*RI site and the start of the 16S rRNA gene (Fig. 2). Type II promoter region, with *Eco*RI site at positon –440, seems to be deletion derivative of the type I and lacks the DNA region including P2 and P3 promoters (Fig. 2). P2 and P4 promoters in type I DNA are identical over 20 bp. This long direct repeat was most probably the cause of deletion of DNA fragment encoding P2 and P3 promoters, as was found in type II DNA. Aside of the internal deletion, two types of promoter regions are highly homologous. However, high homology with the promoter region of *rrnF* is present only in the last 280 bp of DNA, starting from the conserved P4 promoter (Fig. 2).

We have compared putative –10 boxes of P1-P4 promoters of *S. rimosus* rRNA operons with all experimentally identified or proposed promoters of *Streptomyces* rRNA operons found in the literature. The results are summarised in Table 1. In the case of P2 and P4 (identical) promoters sequence identity is extended for several nucleotides on both sides of the –10 boxes. However, sequences of putative –35 sites vary considerably and are often poorly conserved (data not shown).

We cannot claim that three/two ribosomal RNA operons in *S. rimosus* have identical type I/type II promoter regions. We also do not know which of the five remaining rRNA operons (A-E) sequenced type I or type II DNA belongs to. Unfortunately, our cloning procedure resulted in only one positive assay for each of two different *Eco*RI fragments. However, identical sequences from additional clones will never exclude the possibility that we have cloned and sequenced the same DNA repeatedly. Small changes in DNA sequence may exist within three type I and two type II promoter regions. Nevertheless, it is most likely that three rRNA operons in *S. rimosus* are under the control of as much as four promoters (P1, P2, P3 and P4), two operons are expressed from two promoters (P1 and P4) and only the *rrnF* is expressed from one promoter (P4). P4, the most proximal promoter, is the only promoter common to all six *S. rimosus* rRNA operons.

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Operoni za ribosomske RNA bakterije *Streptomyces rimosus*: primarna struktura operona *rrnF* i usporedna analiza promotorskih regija operona za rRNA

Sažetak

U cijelosti je sekvencioniran jedan od šest operona za ribosomske RNA bakterije *Streptomyces rimosus*, nazvan *rrnF.* Geni za rRNA, organizirani u slijedu 16S-23S-5S, kodiraju redom 1529, 3121 i 120 nukleotida duge rRNA. Geni za tRNA nisu nađeni u području između gena za 16S i 23S RNA kao niti na 3' kraju operona. Komparativnom analizom slijeda nukleotida identificiran je samo jedan potencijalni promotor operona *rrnF*, P4. Otvoreni okviri čitanja, smješteni na obje strane operona *rrnF*, sačuvani su na istim položajima i u nekih operona za rRNA u drugih streptomiceta.

Procjenjujući prema sačuvanim *Eco*RI mjestima, preostalih pet operona za rRNA u vrste *S. rimosus* imaju samo dva različita tipa promotorskih regija. Tri operona imaju promotorsku regiju tipa I, a sekvencioniranjem jednog predstavnika utvrđeno je postojanje čak četiri potencijalna promotora (P1-P4). Promotorska regija tipa II, prisutna u dva operona, najvjerojatnije je posljedica interne delecije u tipu I, te nema promotore P2 i P3. Samo su promotor P4 i slijed nukleotida nizvodno od njega jako sačuvani u sva tri tipa promotorskih regija operona za rRNA u bakterije *S. rimosus.*