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Promoters of *Mycoplasma capricolum* ribosomal RNA operons: identical activities but different regulation in homologous and heterologous cells

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ABSTRACT

The 5' region of the rRNA operon, rrnA, of M. capricolum was cloned. Sequence analysis revealed two tRNA genes, $tRNA^{leu}$ and $tRNA^{lys}$, upstream to the promoter of the rRNA operon. The in vivo transcription start sites of the rRNA operon and of the tRNA genes were mapped. The same promoters used by M. capricolum RNA polymerase are also recognized by E. coli RNA polymerase both in vivo and in vitro. We find that high levels of ppGpp in E. coli, resulting from amino acid starvation or from spoT mutation, activate rather than repress the transcription of the mycoplasma rrnA operon.

INTRODUCTION

Mycoplasmas (Class Mollicutes) are wall-less prokaryotes having the smallest genome among self-replicating organisms (1). All the Mollicutes species tested so far carry only one or two ribosomal RNA (rRNA) operons (1,2) compared to seven rRNA operons in Escherichia coli (3) and ten in Bacillus subtilis (4). Transcription of rRNA operons in E. coli is under negative stringent control: amino acid starvation activates the synthesis of guanosine 5'-diphosphate-3'-diphosphate (ppGpp). The accumulation of ppGpp represses the transcription of the rRNA operons (5,6). Transcription of the rRNA operons in E. coli starts from two tandem promoters (3,7). The upstream promoter (P1) is active in cells during high growth rates and is repressed during amino acid or carbon starvation. The downstream promoter (P2) is a constitutive weak promoter, and appears to function as a "maintenance" promoter (8,9). The mechanisms involved in the regulation of transcription of this complex control region are not clear as yet.

M. capricolum was shown to carry two rRNA operons, rrnA and rrnB (10,11). This mycoplasma has also been shown to employ a stringent control mechanism homologous to that of E. coli, as amino acid starvation resulted in the accumulation of ppGpp and decreased synthesis of stable RNA in this organism (12). The mycoplasmas, being parasites, do not usually face sudden changes in environmental conditions. It could be speculated that this would result in a less complex control mechanism for stable RNA synthesis than in E. coli. The fact that mycoplasmas carry only one or at most two rRNA operons (1,2,13) would also be expected to facilitate the study of the control mechanisms of transcription of these genes.

In the present communication we report on the structural and transcriptional analysis of the 5' region of the rrnA operon. This region was sequenced and two tRNA genes were found upstream to the promoter of the rRNA operon. The transcription start sites of the rRNA operon and of the tRNA genes were mapped. Transcription of these genes by E. coli RNA polymerase was studied in vitro and in vivo under different growth conditions. The initial steps of our study have been reported in a preliminary form (14).

MATERIALS AND METHODS

Organisms and Growth Conditions

Mycoplasma capricolum (California kid strain, ATCC 27343) was grown at 37°C for 20-24h to mid-log phase in a modified Edward medium (15) supplemented with 3% (v/v) horse serum. The organisms were harvested by centrifugation at 12,000 xg for 30 min in the cold, and washed once in cold 0.25M NaCl containing 0.1M EDTA. The washed cell pellets were kept at -20°C until DNA extraction. Escherichia coli CF 747 and CF 748, a relA and relA$^+$ isogenic pair of strains (8), and E. coli spoT mutant strains 889 and 890, as well as the isogenic spoT+ strain (16) were obtained from M. Cashel (National Institutes of Health, Bethesda, MD).

DNA Extraction, Restriction and Ligation

Mycoplasmal DNA was extracted according to Marmur (17). Plasmid isolation, restriction cleavage, electrophoresis and cloning techniques were carried out by the standard procedures described in Maniatis et al. (18). Lambda EMBL3 DNA was purchased from Promega Biotec, Madison, WI, USA. Library

construction was performed according to the manufacturers' protocol. Selection of recombinants was done as described by Maniatis et al. (18).

RNA Purification

RNA was purified from E. coli according to the procedure described by Salser et al. (19). Cells were grown at 37°C in 125 ml LB medium (18) to an OD_{600nm}=0.7, poured onto ice, collected by centrifugation and resuspended in 10 ml of 10mM Tris-HCl, pH 7.3, containing 10mM KCl, 5mM $MgCl_2$ and 3 mg lysozyme. The cells were then lysed by freezing (-70°C) and thawing at 64°C. Following addition of 0.7ml of 10% sodium dodecyl sulfate (SDS) solution, the suspension was incubated for 2 min at 64°C. Sodium acetate (pH 5.2) was added to a final concentration of 0.1M, followed by two extractions with water-saturated phenol (pH 4.0) at 64°C. The RNA in the aqueous phase was precipitated with ethanol and the moist pellet was resuspended in 5ml of deionized water and dialyzed extensively at 4° against 0.1mM EDTA.

To isolate M. capricolum RNA, the organisms were grown in one liter of modified Edward's medium and harvested as described above. The pellet was resuspended in a 0.1M sodium acetate solution (pH 5.2) containing 0.1M EDTA, and frozen at -70°C. RNA extraction was carried out as described above for E. coli, except that sodium acetate was not added before the phenol extractions.

Small scale RNA isolation from amino-acid starved E. coli cells was carried out according to Sarmientos et al. (8). The organisms were grown at 37°C in LB medium. When cell density reached an OD_{600nm} of 0.2-0.3, DL-serine hydroxamate (Sigma Chemical Co., St. Louis, MO, USA) was added to 1mg/ml. A 2ml culture aliquot was then transferred into a glass tube containing 0.5ml of 1M NaCl, 25% SDS and 50 mM EDTA (pH 8.0) and held in a boiling water bath for 10-15 sec. The resulting cell lysate was cooled to room temperature, extracted with phenol and the RNA precipitated with ethanol. The moist pellet was dissolved in 0.4ml of 0.3M sodium acetate (pH 5.0) and used for primer extension analysis as described below.

Primer extension analysis

E. coli or M. capricolum RNA (100 µg), or total RNA of a small-scale preparation from a 2 ml culture, were mixed with 40ng of 5'-end-labeled oligonucleotide primer synthesized

according to the phosphoamidite method (20) and precipitated with ethanol. The precipitate was resuspended in 45µl of 0.1M Tris-HCl, pH 8.3, 0.14 M KCl and 0.01M $MgCl_2$. The mixture was incubated at 65°C for 10 min and cooled at room temperature for 5 min. Dithiothreitol was added to a final concentration of 0.01M and the four deoxynucleotide triphosphates to a final concentration of 1mM. Reverse transcriptase (18 units) of AMV (International Biotechnologies Inc, New Haven, Conn., USA) was added and the mixture was incubated at 42°C for 1h. The reaction was then stopped by heat inactivation at 75°C for 10 min. DNase-free RNase (1µg) was added and incubation continued for 30min at 37°C. Ammonium acetate was added to 0.4M followed by ethanol precipitation overnight at -20°C. The precipitate was resuspended in 98% formamide, 0.1% bromophenol blue, 0.1% xylan cyanol, 2mM EDTA and run on a sequencing gel.

In vitro transcription

This was carried out as described before (21). E. coli RNA polymerase was the product of Pharmacia Biotechnology International (Uppsala, Sweden).

DNA sequencing

Sequence analysis was done by the Sanger dideoxy method (22) using ordered deletions generated by exonuclease III digestion (23) and, sequencing kits bought from Amersham (England) and International Biotechnologies Inc. (New Haven, Conn., USA). Computer analysis of the DNA sequences was performed with the Genetic Computer Group Sequence Analysis Software Package (24).

RESULTS

Cloning of the 5' part of the rrnA operon of M. capricolum

A library containing M. capricolum chromosomal DNA, partially cut by SauIIIA, was prepared by us (14) in the lambda EMBL3 vector (25). Clones carrying rDNA sequences were identified by plaque hybridization with pMC5, a probe carrying the major part of the rrnA operon of M. capricolum (11). Restriction mapping and hybridization analysis with probes from the 5' and 3' regions of the rrnB operon of E. coli (11) revealed that clone 21 carried the entire rrnA operon of M. capricolum (Fig. 1). The 3.0kb PstI and the 1.3 kb BglII fragments, containing part of the 16S rRNA gene and 5' flanking

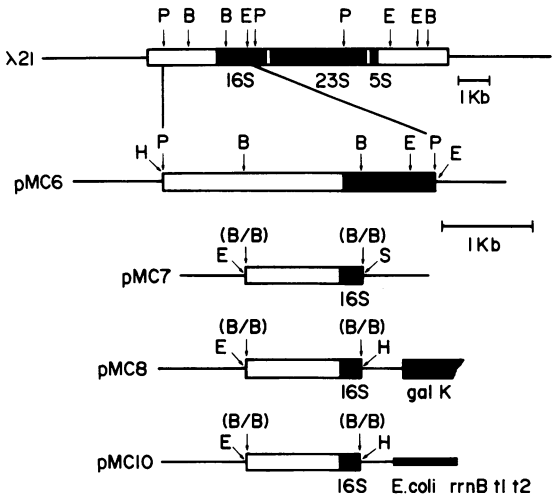

Figure 1. Clones of the 5' region of the rrnA operon of M. capricolum in different vectors. Restriction sites: B - BglII, E - EcoRI, H - HindIII, S - SalI, P - PstI, (B/B) - ligation of BglII donor to BamHI acceptor site.

sequences were subcloned into pUC18 vector to give subclones pMC6 and pMC7, respectively (Fig. 1). The 1.3kb BglII fragment was also subcloned into the pGC14 (26) vector, upstream to a promoter-less galK gene (subclone pMC8, Fig. 1) and upstream to rRNA terminators of E. coli rrnB in the vector pPS3ML (subclone pMC10, Fig. 1). pPS3ML is a vector derived from pps1 (8) by deleting the promoter and inserting mp8 multilinker at the EcoRI-HindIII sites. The various subclones were used for in vitro and in vivo transcription experiments.

Sequence analysis of the 5' region of the rrnA operon

The sequence of 700 bp out of the 1.3kb BglII fragment of pMC7 is presented in Fig. 2. The sequence includes the first 174 bases of the 16S rRNA gene and 531 bases upstream to the gene. The first nucleotide of the mature 16S rRNA is designated +1 (Fig. 2). Analysis of the nucleotide sequence revealed two tRNA genes located upstream to the 16S rRNA gene. These are genes for $tRNA^{lys}$ (nucleotides -438 to -363) and $tRNA^{leu}$ (nucleotides -360 to -278).

In vitro transcription of rrnA

Transcription products of the different subclones by E. coli RNA polymerase are shown in Fig. 3. Transcription of pMC8 cut by HindIII gave two transcripts of about 370 and 780 bases

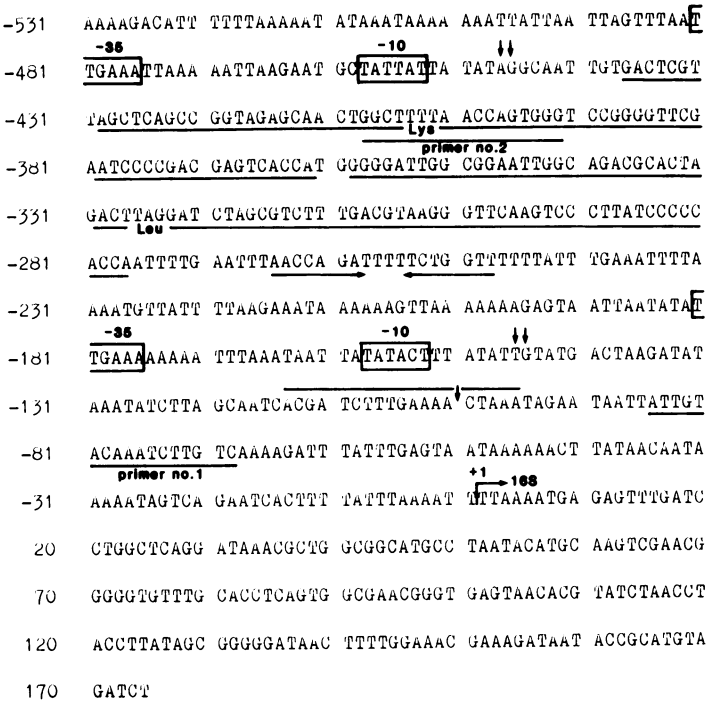

Figure 2. The nucleotide sequence of the 5' end of the rrnA operon. The non-coding, RNA-like strand is shown. The nucleotides are numbered from the start site of the 16S rRNA gene, designated as +1. Mapped start sites are marked by arrows. Putative -10 and -35 sequences are boxed. The nucleotides of the two tRNA genes are underlined with the amino acid designation inserted at the anticodon site. Oligonucleotide primers no. 1 and no. 2, used for primer extension are underlined. The 16S rRNA processing sequence is marked by a line above the sequence, with an arrow pointing at the cleavage site. A sequence capable of forming a stem and loop and possibly functioning as a termination signal is marked by confronting arrows.

long (Fig. 3, lane 4). Transcription of the same plasmid (pMC8) cut by EcoRI gave no transcripts (Fig. 3, lane 5). The vector pGC14 with no insert gave no detectable transcripts when cut by either HindIII or EcoRI (Fig. 3, lanes 2 and 3). When subclone pMC6 cut by BglII was used as a template for in vitro transcription (Fig. 3, lane 6), the same two transcripts were detected as with pMC8 (370 and 780 bp). When cut by EcoRI, plasmid pMC6 gave two longer transcripts of about 900 and 1300 bases (Fig. 3, lane 7). pMC6 cut by HindIII gave no visible transcripts (data not shown). The in vitro

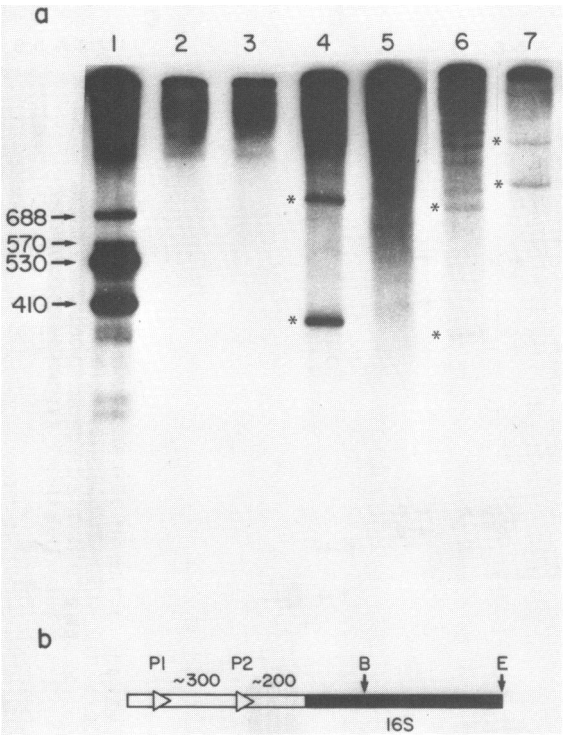

Figure 3 a) In vitro transcription of cloned fragments of the 5' end of the rrnA operon by E. coli RNA polymerase. Lane 1, transcripts of pPS1 (8) used as size markers; lanes 2 and 3, transcription control of the vector pGC14 (26) cut by HindIII or by EcoRI, respectively; lane 4, pMC8 cut by HindIII, showing the two transcripts (marked by asterisks); lane 5, pMC8 cut by EcoRI, showing no transcripts in this direction; lane 6, pMC6 cut by BglII, showing the same two transcripts as in lane 4 (marked by asterisks); lane 7, pMC6 cut by EcoRI, showing two longer transcripts (marked by asterisks) beginning at the same start sites as those shown in lanes 4 and 6.
b) A scheme showing the approximate position of the promoters in relationship to the 16S rRNA gene.

transcripts shown in Fig. 3 were run on a SDS-acrylamide gel. It appears that the longer transcript on SDS-gels, which contains two tRNAs, runs more slowly than expected from its size, as SDS is not always effective in completely denaturing RNA, particularly when it contains a rigid secondary structure characterizing tRNAs. In fact, the size of the longer transcript, when measured on the sequencing gel containing urea, a more effective denaturing agent than SDS, was only about 600

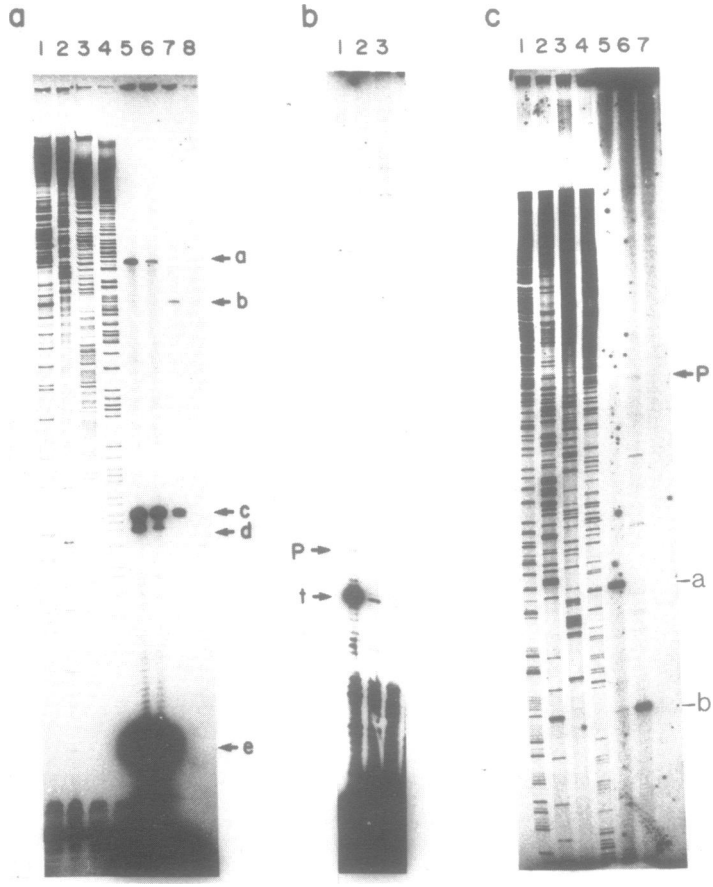

Figure 4 a) Primer extension analysis of the rrnA promoter region using the oligonucleotide primer no. 1 (Fig. 2). Lanes 1-4, sequencing reactions (A,C,G and T respectively) produced using the same primer and the promoter region cloned in an M13 vector as a template. Lane 5 - RNA of M. capricolum, chloramphenicol was added to these cells 30 min prior to harvesting; lane 6 - the same as lane 5 but the organisms were grown without chloramphenicol; lane 7 - RNA from E. coli cells carrying pMC10; lane 8 - RNA from E. coli cells carrying pPS3ML.
b) Primer extension analysis of the region upstream to the two tRNA genes, using primer no. 2 (Fig. 2). Lane 1, M. capricolum RNA; lane 2, RNA from E. coli carrying pMC10; lane 3, RNA from E. coli carrying the vector pPS3ML.
c) Identification of the tRNA operon promoter using primer no. 1. Lanes 1-4 same as in (a), lane 5 - RNA of M. capricolum, lane 6 - RNA from E. coli cells carrying pMC10; lane 7 - RNA from E. coli cells carrying pPS3ML. This gel was run longer than that in (a) so that longer transcripts could be identified. In addition, the exposure time of the X-ray film was 6 weeks. Bands a and b, shown in Fig. 4a are also indicated on this part of the figure.

bases long. This suggests that the distance between the two transcription start sites is about 300 bases, a finding in line with the data obtained in the in vivo transcription experiments (see below). The interpretation of these in vitro transcription data is that the E. coli RNA polymerase recognizes two promoters on the BglII fragment of the M. capricolum rrnA operon. The promoters are located about 200 and 500bp upstream to the 5' end of the 16S gene (Fig. 3b). These two promoters induce transcription in the direction of the 16S gene, as judged from the different transcripts of the subclones digested by the various restriction enzymes. The orientation of the BglII fragment on the subclones was verified by sequencing the 16S gene from the HindIII site of pMC8 and by restriction analysis of pMC6.

In vivo transcription of rrnA in M. capricolum and in E. coli

Plasmid pMC8 which carries the 5' end of the rrnA operon upstream to galK gene, was introduced to a galK⁻ E. coli strain. When this transfected strain was grown on Gal-McConkey agar plates, the colonies appeared red, indicating that transcription originating from the mycoplasmal rrnA fragment into the galK gene occurs also in vivo, as well as in vitro. To identify transcription start sites and processing sites of rrnA, primer extension experiments were performed, using RNA isolated from M. capricolum and from E. coli carrying pMC10. The sequence of the oligonucleotide primer used (primer no. 1, Fig. 2) is common to the two rRNA operons (rrnA and rrnB) of M. capricolum (27) and is located upstream to the 5' end of the mature 16S rRNA. With M. capricolum RNA, five major bands representing different 5' ends were identified (bands a-e, Fig. 4a, lanes 5 and 6). No difference was observed when chloramphenicol was added to the cells prior to RNA extraction. With RNA of E. coli carrying pMC10 as template only bands b and c appeared (Fig. 4a, lane 7). RNA from E. coli carrying the vector pPS3ML gave no primer extension products (Fig. 4a, lane 8). The primer extension reaction products were run alongside the sequence analysis using the same primer in order to identify the exact location of the 5' ends (Fig. 4a, lanes 1-4).

The following identification of the RNA species detected by the primer extension experiments is proposed: transcript e is the first precursor of the 16S rRNA. The 5' end of this

precursor results from cleavage within a conserved processing site sequence (underlined in Fig. 2). Transcript c (Fig. 4, lanes 5-7), apparently represents the start site of the rrnA transcript. This band is a doublet and the start sites are indicated in Fig. 2. Typical -10 and -35 regions, resembling the E. coli promoter consensus sequences, are found very close upstream to the start site (boxed in Fig. 2). The same start site (doublet band c) is also found in E. coli carrying the rrnA promoter region in plasmid pMC10 (Fig. 4a, lane 7) indicating that the E. coli RNA polymerase recognizes in vivo the same promoter used by the mycoplasma. Since bands a and d (Fig. 4, lanes 5 and 6) appeared only with the M. capricolum RNA template these bands presumably represent transcription start sites of the second rRNA operon of this organism, operon rrnB. Band b (Fig. 4a, lanes 5-7) falls in a stem and loop structure found between the tRNA genes and the rrnA promoter. We suggest that this band results from detachment of the reverse transcriptase on encountering the secondary structure on the RNA. If correct, it means that a transcript starting at the tRNA promoter is read through the rrnA operon.

To determine the transcription start site of the two tRNA genes found upstream to the rrnA operon, we used a second primer, primer no. 2 (Fig. 2). The primer extension experiment yielded a strong band representing the 5' end of the mature $tRNA^{lys}$ (Fig. 4b, lane 1, band t) and a run of weaker bands upstream to the major band. Since most of the primer hybridized with the mature tRNA, it was difficult to identify the transcription start site. We assume that band p (Fig. 4b, lane 1) represents the 5' end of the transcript. The start sites and the -10 and -35 regions upstream to the tRNA genes are marked on Fig. 2. When RNA from E. coli-carrying pMC10 was used as a template, the same transcription start site was identified as with the mycoplasmal RNA (Fig. 4b, lane 2, band p) as well as a small amount of mature tRNA (band t). These two bands were not detected in a control experiment using RNA from E. coli cells carrying the pPS3ML vector alone (Fig. 4b, lane 3).

A stem and loop structure followed by a run of T's is found in the spacer region between the $tRNA^{leu}$ and the rrnA promoter (Fig. 2). This terminator-like structure and the strong promoter found upstream to the 16S rRNA gene (Fig. 4a, lane 5,

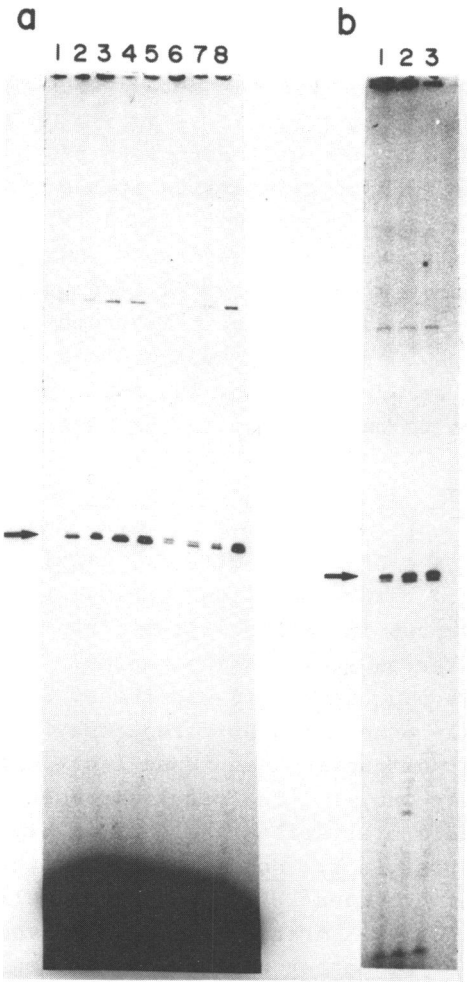

Figure 5. Expression of the mycoplasmal rrnA promoter in E. coli in the presence of various levels of ppGpp. Serine hydroxamate to a final concentration of 1mg/ml was added to the culture at OD_{600} of 0.2-0.3. a) Lanes 1-4, E. coli CF748 (relA$^+$) carrying pMC10 at 0,2,4, and 8 minutes after addition of serine hydroxamate; lanes 5-8, the same as 1-4 but E. coli CF747 (relA-) carrying pMC10.
b). Three E. coli strains carrying pMC10 were grown to an OD_{600}=0.2-0.3, and their RNA was extracted and used for primer extension. Lane 1 wild type CF887 (spoT$^+$); lane 2, strain CF890 (spoT 202); lane 3, strain CF889 (spoT 204).

band c), suggest that the two tRNA genes and the rRNA genes constitute two separate transcription units. To examine whether there is a transcription readthrough from the tRNA promoter to

the rRNA operon, a primer extension reaction using primer no. 1 and M. capricolum RNA were used. No band representing the tRNA promoter start site was detected even after 6 weeks exposure of the X-ray film (Fig. 4c, lane 5). On the other hand, when RNA extract from E. coli carrying pMC10 was used, a band representing a transcript starting at the tRNA promoter and extending into the rrnA operon was found (Fig. 4c, lane 6). The in vitro transcription experiments using the E. coli RNA polymerase described above (Fig. 3) also support the finding of transcription originating in the tRNA promoter and continuing into the rRNA operon. Our failure to show this with mycoplasmal RNA, can possibly be explained by the fast processing of the long RNA transcript to tRNA's, and to the rRNA precursor in the mycoplasma cell.

Stringent control of the rrnA promoter in E. coli

Since the E. coli RNA polymerase was shown to recognize and use the rrnA promoter of M. capricolum both in vivo and in vitro, the question was whether E. coli is also capable of repressing transcription from the cloned rrnA promoter when starved for amino acids. E. coli cells containing pMC10 were grown to mid-log phase. Serine hydroxamate was added to induce the stringent response. At several time intervals RNA was extracted and used for primer extension reaction with primer no. 1. The results presented in Fig. 5a show that amino acid starvation resulted in higher amounts of the rrnA transcripts in E. coli within 2 min after serine hydroxamate addition. When E. coli relA cells were used the elevated level of the transcript was observed only after 8 min probably because of carbon starvation induced by the serine hydroxamate (6). It thus appears that transcription through the M capricolum rrnA promoter is activated rather than repressed by ppGpp in the E. coli cells.

To further investigate the effect of ppGpp on transcription of the mycoplasma rrnA promoter in E. coli we used spoT mutants. The product of the spoT gene is responsible for ppGpp degradation, so that spoT cells have higher basal levels of ppGpp than the wild type (16). A primer extension experiment showed that the amount of rrnA transcripts in E. coli spoT cells carrying pMC10 was higher than in wild type cells (Fig. 5b).

DISCUSSION

To facilitate our understanding of the control of stable RNA synthesis in prokaryotes we have sequenced the 5' upstream region of the rrnA operon of M. capricolum and analyzed transcription of this operon in this mycoplasma and in E. coli. Sequence analysis of the cloned DNA fragment containing the above region revealed two tRNA genes, $tRNA^{lys}$ and $tRNA^{leu}$, upstream to the 16S gene. Similar sequencing data of the 5' end of the rrnA operon of M. capricolum were recently and independently obtained by C. Taschke and R. Herrmann (private communication). The sequence of the homologous region of rrnA from Mycoplasma sp. PG50, a mycoplasma closely related to M. capricolum, has a 99.6% homology with the M. capricolum sequence, including the tRNA genes (28).

A recognition sequence for processing the primary rRNA transcript was identified in position -115 to -97 (Fig. 2). This sequence is similar to the processing signals found in B. subtilis (29) and in M. hyopneumoniae (13), and is capable of base pairing with an inverted repeat found in the spacer region between the 16S and the 23S rRNA genes; the stem structure formed in this way serves apparently as a recognition site for a processing enzyme, as described for E. coli (30) and B. subtilis (31). The exact position of the 16S rRNA precursor resulting form cleavage at this site is indicated in Fig. 2.

The start for the rrnA transcription is 146-147 nucleotides upstream to the 16S rRNA 5' end. The transcription start of the two tRNA genes is 9-10 nucleotides upstream to the 5' end of the $tRNA^{lys}$. The two transcription start sites are preceded by -10 and -35 boxes, exhibiting good agreement to the consensus sequences of E. coli promoters (32). Two additional transcription start sites, indicated by our primer extension experiments (Fig. 4a) are apparently associated with the second rRNA operon of M. capricolum, operon rrnB. The sequence and the transcription analysis of this operon (C. Taschke and R. Herrmann, private communication) suggested the presence of two tandem promoters 150 nucleotides apart with no tRNA genes in between. The sequences of the rrnB promoters also resemble the consensus sequence of E. coli promoters. On the other hand, for the single rRNA operon of M. pneumoniae only one promoter with a typical consensus sequence

has been identified, though a second start point further upstream, having no resemblance to a consensus sequence has also been suggested (H. Hyman, R. Gafny, G. Glaser, S. Razin, submitted for publication).

The presence of two tandem promoters for rRNA operons was reported for E. coli (7), B. subtilis (33) and M. hyopneumoniae (13). Although the rrnB operon of M. capricolum may also conform to this scheme in having two tandem promoters (see above), the rrnA operon appears to have a different structure as two tRNA genes are located upstream to the single promoter definitely shown to be associated with transcription of the rrnA operon. We have identified a readthrough transcription from the tRNA promoter into the rRNA operon by E. coli RNA polymerase in vitro and in vivo, but we could not identify such a transcript in RNA extracted from M. capricolum. It is possible that this transcript could not be detected because of its fast processing into mature tRNA and rRNAs. The finding of a primer extension band upstream the rrnA promoter in M. capricolum and in E. coli carrying pMC10 (band b in Fig. 4a) supports a transcription readthrough. On the other hand the stem and loop structure followed by a run of T's found between the tRNA genes and the ribosomal RNA promoter, suggests that at least some of the transcripts originating from the tRNA promoter terminate before reaching the rrnA operon. At the present we cannot prove or disprove the existence of a transcription readthrough from the the tRNA promoter to the rrnA operon in M. capricolum.

As in E. coli, rRNA synthesis in M. capricolum is subject to negative stringent control expressed by the accumulation of ppGpp and repression of rRNA synthesis on amino acid starvation (12). According to our present findings, E. coli RNA polymerase recognizes in vivo the same promoter used by M. capricolum for transcription of rrnA and the transcription start site is the same in both organisms. We have found that a high level of ppGpp in E. coli, resulting from amino acid starvation or from spoT mutation, activated rather than repressed the transcription of the mycoplasma rrnA promoter. The sequence characteristics that activate or repress an E. coli promoter by ppGpp are not yet clear. It was suggested that a GC rich "discriminator" sequence between position -7 to +1 (34) and the dinucleotide AA in the fourth and

fifth position of the -10 box (35) are the features required for supression of an E. coli promoter by ppGpp. It can be suggested that since the M. capricolum rrnA promoter lacks these features ppGpp activates rather than supresses transcription.

Point mutations in the beta-subunit of the E. coli RNA polymerase can result in a relaxed phenotype (36). On the other hand, some spontaneous rifampin-resistant mutations in the beta-subunit were shown to result in hypersensitivity to ppGpp levels (37). These mutation experiments suggest that the beta-subunit of RNA polymerase is the target of ppGpp transcription regulation. Growth of all the Mollicutes species tested so far is not inhibited by rifampin(1). It was recently shown that purified RNA polymerase from spiroplasma strains is resistant to rifampin inhibition of transcription (38). This indicates that mycoplasma and E. coli polymerases differ in their beta-subunit structure, the target for rifampin action. The difference in the beta-subunits may be responsible for the finding that E. coli RNA polymerase recognizes the mycoplasmal rRNA promoter as a promoter activated by ppGpp, whereas in the mycoplasma this promoter is apparently inhibited by ppGpp.

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