Transcriptional Analysis of the 16S rRNA Gene in *Rickettsia prowazekii*

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The control of rRNA synthesis in the etiological agent of epidemic typhus, *Rickettsia prowazekii*, a slowly growing obligate intracytoplasmic bacterium, was investigated. Transcription of the rickettsial 16S rRNA gene (*rrs*), of which there is only a single copy, was controlled by a single promoter region, and the site for the initiation of transcription (base A) was found 117 bp upstream of the *rrs* coding region for the mature product. The promoter region contained an *Escherichia coli* promoter-like sequence, TTGACA-N₁₇-TATAAC, centered 139 bp upstream of the coding region for the mature product. To investigate whether transcription of the rickettsial *rrs* responds to amino acid starvation conditions, total RNA was isolated from *R. prowazekii*-infected mouse L929 cells with or without methionine starvation. The level of newly synthesized 16S rRNA precursors in *R. prowazekii*, as analyzed by ribonuclease protection assays, decreased significantly after methionine starvation for 6 h and then recovered within 12 h after the addition of methionine. The chemical half-lives of the 16S rRNA precursors in the methionine-starved rickettsiae did not differ significantly from those in the normal rickettsiae. These results suggest that *R. prowazekii* regulates transcription of the *rrs* in response to amino acid starvation conditions.

Rickettsia prowazekii, the etiological agent of epidemic typhus, is an obligate intracytoplasmic bacterium with a typical gram-negative bacterial morphology (24, 25). The growth rate of the rickettsiae (about 10 h per generation) (26, 27) is considered low in comparison with the growth rates of facultative bacteria. In general, the rate of rRNA synthesis determines the rate of ribosome synthesis, is responsive to the nutritional environment, and is under growth rate-dependent control (9, 10, 14). In *Escherichia coli*, seven redundant rRNA (*rrn*) gene operons, two tandem promoters for each of the operons, and A/T-rich upstream sequences are considered largely responsible for the high rate of rRNA transcription (10). The *R. prowazekii* genome has only one 16S rRNA gene (*rrs*) and only one 23S rRNA gene, and they are at distinct loci (1, 16). No information on the upstream sequence and the control of 16S rRNA synthesis in members of the genus *Rickettsia* was available prior to this study, although the sequence of the mature 16S rRNA had been determined by PCR analysis (23).

In the present study, we cloned, sequenced and functionally characterized the upstream promoter region of the rickettsial *rrs*. We also investigated whether *R. prowazekii* controls 16S rRNA production in response to amino acid starvation. An understanding of the control of rRNA production could provide valuable insight into global regulatory mechanisms in these obligate intracytoplasmic parasites.

MATERIALS AND METHODS

Construction of plasmids. The upstream region and 5' end of the coding portion of the rickettsial *rrs* was cloned into pBluescript SK (Stratagene, La Jolla, Calif.) from the genomic DNA of *R. prowazekii*. The rickettsial genomic DNA was isolated from rickettsiae growing in chicken embryonic yolk sacs as described previously (16) and was digested with restriction enzymes *Xba*I and *Sma*I

(GIBCO-BRL Life Technologies, Inc., Gaithersburg, Md.). After electrophoresis, the DNA fragments in the region between 0.5 and 2 kb were eluted from the gel with GeneClean II Kit (Bio 101 Inc., La Jolla, Calif.) and were cloned into the multicloning sites of pBluescript SK. The resulting plasmid, pHW42 (Fig. 1), containing the approximately 1.2-kb upstream region as well as 579 bp of the 5'-coding region of the gene, was verified by Southern blotting with a DNA probe which could hybridize to the 5' end of the coding region of the gene (16) and by DNA sequencing. Plasmid pHW43 containing the *Xba*I-*Sst*I fragment of the rickettsial *rrs* (Fig. 1) was generated by inserting this fragment of pHW42 after partial digestion into the *Sst*I site of a modified pBluescript vector (removing the *Not*I-*Eco*RV fragment). Plasmid pHW44, which contains the 1.2-kb upstream region and 91 bp of the 5'-end-coding portion of the rickettsial *rrs*, was constructed by replacing the *Xba*I-*Cl*aI fragment of pHW11 (6) with the *Xba*I-*Cl*aI fragment of pHW43. Plasmid pHW45 was constructed by cloning a 480-bp fragment (containing the region from -47 to -526), which was amplified (16) by PCR with forward primer 1 (5'-CATGCGGAGACAGATTC-3') and reverse primer 2 (5'-GTACATTTTACTGTAC-3') with pHW44 as the template, into the TA vector (Invitrogen Corp., San Diego, Calif.) (Fig. 1). Plasmid pHW47 was constructed from pHW44 by removing the *Hinc*II-*Cl*aI fragment (Fig. 1).

DNA sequencing. DNA sequencing was performed by the dideoxynucleotide chain termination method described by Sanger et al. (21) with modified T7 DNA polymerase according to the instructions of the manufacturer (Sequenase, U.S. Biochemicals, Cleveland, Ohio). Long Ranger (J. T. Baker, Phillipsburg, N.J.) was used to prepare DNA sequencing gels according to the manufacturer's instructions.

Isolation of total RNA from the rickettsiae-infected mouse L929 cells. Total RNA, a mixture of host cell total RNA and rickettsial total RNA, was isolated from rickettsiae-infected mouse L929 cells (Flow Laboratories, Inc. McLean, Va.) by the hot-phenol method as described previously (3). RNA in the aqueous phase was precipitated with ethanol and stored at -80°C.

RNAse protection assays. Specific antisense RNA probes labeled with [α -³²P]UTP (ICN Biomedicals, Inc., Irvine, Calif.) were generated by in vitro runoff transcription with T7 RNA polymerase (RNAP) according to the manufacturer's instructions (Promega Corp., Madison, Wis.). Probe 1 (606 nucleotides [nt] in length) contained 480 bp of the region (-47 to -526) upstream of the rickettsial *rrs* and 126 bp of unrelated vector sequence and was generated with *Hind*III-linearized pHW45 as the template (Fig. 2). Probe 2 (617 nt in length) contained 525 bp of the *rrs* coding region (the *Sst*I-*Xba*I fragment) and 92 bp of unrelated vector sequence and was generated with *Sst*I-linearized pHW40 (16) as the template (Fig. 2). RNase protection assays were performed according to the standard method (20). Total RNA (100 μ g) was hybridized with approximately 10⁶ cpm of the probes. The temperatures of hybridization were 30°C for probe 1, which hybridized only to the newly synthesized precursors of 16S rRNA, and 40°C for probe 2, which hybridized to both the newly synthesized precursors and preexisting 16S rRNA. After hybridization, the samples were digested with RNase A (40 μ g/ml) (Sigma Chemical Co., St. Louis, Mo.) at 37°C for 60 min. The reaction was terminated by the addition of sodium dodecyl sulfate (SDS) (to

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FIG. 1. Cloning the upstream sequence of the rickettsial *rrs*. pBluescript SK was used as the vector to construct pHW42 and pHW43. pHW11 was used as the vector to construct pHW44 and pHW47. TA vector was used as the vector to construct pHW45. Dark segments, the coding region for the mature 16S rRNA; hatched segments, upstream region of the rickettsial *rrs*; cross-hatched segments, transcriptional terminator; lines, vector parts of the plasmids. The two arrowheads under pHW44 represent primers used to generate a DNA fragment by PCR for construction of pHW45. (H/C), the site after removal of the *HincII*-*ClaI* fragment and religation of pHW44.

0.5%) and proteinase K (to 4 mg/ml), and the samples were further incubated at 37°C for 20 min. The samples were then precipitated with ethanol, electrophoresed in 6% polyacrylamide-urea (7 M) gels, and analyzed by autoradiography. The sizes of the protected bands were estimated with DNA size markers (GIBCO-BRL Life Technologies, Inc. Gaithersburg, Md.).

In vitro runoff transcription assays. The assays for specific transcription were performed as described previously (6). Briefly, a reaction mixture containing 40 mM Tris-HCl (pH 7.9), 10 mM MgCl₂, 1 mM dithiothreitol, 2 mM spermidine-HCl, 50 mM NaCl, 100 µg of bovine serum albumin per ml, and 50 U of RNasin (Pharmacia Corp., Madison, Wis.) was prepared. Supercoiled plasmid template (approximately 15 nM) with terminators inserted downstream of the region of interest was preincubated with 150 nM purified rickettsial RNAP (5) in this mixture at room temperature for 20 min. Transcription was then initiated by the addition of a solution containing final concentrations of 1 mM ATP, CTP, GTP, and 0.1 mM UTP and 20 µCi of [α -³²P]UTP. The reaction was carried out in a final volume of 50 µl at 37°C for 30 min and stopped by the addition of an equal volume of stop solution containing final concentrations of 10 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 250 mM NaCl, 0.5% SDS, and 200 µg of proteinase K per ml, and the samples were further incubated at 37°C for 30 min. The transcripts were precipitated with ethanol, separated on a 6% polyacrylamide-urea (7 M) gel, and analyzed by autoradiography. The sizes of the specific transcripts were estimated with DNA size markers.

Primer extension assays. Primer extension assays were performed according to the standard methods (20). Primer 2 (5'-GTACATTCTTACTGTCAC-3'), which is complementary to the -47 to -64 sequence upstream of the coding region, was used for extension. RNA was obtained from both the rickettsia-infected and the mock-infected L929 cells, and in vitro transcript was produced by using pHW44 as the template.

Growth of *R. prowazekii* in mouse L929 cells cultured with and without supplemental methionine. Mouse L929 cells were infected with rickettsiae at a multiplicity of infection of 60, and the rickettsia-infected cells were cultured in

FIG. 2. Generation of antisense RNA probes for RNase protection assays. Antisense RNA probe 1 was generated from *HindIII*-linearized pHW45, and probe 2 was generated from *SstI*-linearized pHW40 with T7 RNAP. Dark segments, coding region of the rickettsial *rrs*; hatched segments, upstream region of the rickettsial *rrs*; lines, vector parts of the plasmids; arrowheads, primers used to generate PCR fragments for construction of pHW45 and pHW40; solid circles, the promoter of T7 RNAP.

methionine-deficient Eagle minimal essential medium (MEM; Mediatech, Washington, D.C.) with 25 µM methionine and 10% dialyzed newborn calf serum (KC Biologicals, Lenexa, Kans.) in a humidified atmosphere of 3% CO₂ in air at 34°C. After incubation for 40 h, the cells were washed thrice with methionine-deficient MEM, and the medium was changed to methionine-deficient MEM with 10% dialyzed newborn calf serum with no supplemental methionine. The infected cells were subsequently cultured in this methionine-free medium. At 12 h after the removal of methionine, methionine at a final concentration of 100 µM was added back to some dishes for another 12 h. Total RNA was isolated at 0, 6, 12, and 24 h after methionine starvation, and RNase protection assays were performed. The density of the probe 1-protected band was employed to determine the relative amounts of the newly synthesized precursors of 16S rRNA in each sample. Before the comparison, the densities of the probe 1-protected bands were normalized to the densities of the probe 2-protected bands (total 16S rRNA) after densitometry analysis to correct for recovery. Coverslips were removed and stained to monitor the growth of the rickettsiae.

Determination of decay of the rickettsial 16S rRNA. To measure the chemical half-lives of the newly synthesized precursors of 16S rRNA in *R. prowazekii*, RNA samples were isolated at various times after the addition of rifampin to infected L929 cells at a final concentration of 0.4 mg/ml to inhibit transcriptional initiation (3). RNase protection assays were performed, the amounts of newly synthesized precursors and total 16S rRNA in each sample were quantitated by densitometric analysis of autoradiograms, and the rates of decay were calculated by least-squares determination of the slope of a logarithmic plot of band density against time.

Nucleotide sequence accession number. The sequence reported here has been deposited with GenBank under accession number U29135.

RESULTS

Cloning and DNA sequence of the upstream region of the rickettsial *rrs*. The initial step to characterize transcription was to clone the upstream region of the gene. An *XbaI*-*SmaI* fragment was targeted for cloning, since an *XbaI* site occurs approximately 1.2 kb upstream of the gene (16), the rickettsial *rrs* contains only one *SmaI* site (23), and there are only six *SmaI* sites in the A/T-rich rickettsial genome (28). pHW42 contained approximately 1.2 kb of upstream sequence and 579 bp of the 5' portion of the coding region of the rickettsial *rrs* (Fig. 1).

The region from position -1 to -533 upstream of the coding region of the rickettsial *rrs* was sequenced (Fig. 3). This was believed to be adequate for transcriptional analysis since in *E. coli* all of the regulatory elements are located within the 500-bp upstream region of the *rrs* (7, 10).

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TTACTGTCATGCGGAAGCACAGATTCAAAGTTATTGAAAGCTGGATCAGA 50
TAAATGATAGAAAAAATAATAAGCAGCAATAATGTGGATTATTTTAAAC 100
TCAGTATCTTGAATTATGCTTGAATAATTTGATGATCCTAGTACTACTC 150
ATTGAGTAGTATTATACTATATAAGGATTTACTAAATCAATAACTAAGA 200
TTAATAGTTTGTTCACGATCTTAATTTTAAAGCTAAGTAAGCTAAATTTT 250
CAATCGTATCCAATTTTTTAATTCATTTTTTTGGAGACACCAAAATC 300
TTATTAATTTTTAGTGATCAATTAATAAGAGCATAACATATTCTTAA 350
TAAAAAATAAAATAATCTCGAAATTTAGTTGACAGGCTGATTATATT 400
-35
GCATATAACTGCAACTATACAAAGCGAAAGTCGCAGCTGTTTTTAAAGT 450
-10 *
AAATAATTAGATATAGATAGTACAGTAAAAATGTACACATCACAAAAGT 500
CACTGTCATTTTACATG
primer 2
GATAATTTACCTGTCAATTTTGAATAAAAGTACAGAAATCAAACTTGA 550

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FIG. 3. Nucleotide sequence of the upstream region of the *rms* of *R. prowazekii*. Boldface sequence beginning at base 534 represents the coding sequence of the *rms*. The promoter elements are indicated at -35 (TTGACA) and -10 (TATAAC). The transcriptional start site (+1) is indicated by an asterisk at base A. The location and sequence of the primer used for primer extension mapping are denoted.

Identification of a functional promoter region responsible for the transcription of the rickettsial *rms* by in vitro transcription analysis. To determine whether there are functional promoters for the rickettsial *rms* within this cloned upstream sequence, in vitro transcription assays were performed with purified rickettsial RNAP and the supercoiled plasmid (pHW44) as the template. This plasmid contains 1.2 kb of upstream sequence, a 91-bp coding region for the mature 16S rRNA, and a *trpA* transcriptional terminator. Supercoiled plasmids were used as templates because purified RNAP of *R. prowazekii* functions very poorly on linear or open-circular templates (5). As shown in Fig. 4, two specific transcripts with sizes of 240 and 310 nt were synthesized by purified rickettsial RNAP as well as by *E. coli* RNAP. No transcripts shorter than 240 nt were observed. On the basis of the sizes of these two in vitro transcripts, the promoter region of the 240-nt transcript should be located at approximately position -120 and that of the 310-nt transcript should be located at approximately position -200 . Indeed, an *E. coli*-like consensus promoter sequence which contained a perfectly matched -35 box, a 17-bp spacer, and a -10 box was found in the upstream region corresponding to the 240-nt

FIG. 4. In vitro transcription of the rickettsial *rms*. Supercoiled plasmids (pHW44 and pHW47) were used as templates. Pol, polymerase; Ec, *E. coli* RNAP; Rp, *R. prowazekii* RNAP. Arrows indicate the specific transcripts.

transcript (Fig. 3). However, no *E. coli*-like consensus promoter sequence could be found in the region corresponding to the 310-nt transcript. This 310-nt transcript may initiate from a promoter which differs entirely from the *E. coli* consensus, or the coding sequence of this transcript may be located upstream of *rms* and hence may be unrelated to the transcription of the *rms*.

pHW47 was constructed to clarify the relationship of the 310-nt transcript and the *rms*. The *HincII*-*ClaI* fragment of pHW44 was removed so that pHW47 did not contain the promoter region and the *rms* coding region of the 240-nt transcript (Fig. 1). If the 310-nt transcript is relevant to 16S rRNA transcription, then removal of this downstream *HincII*-*ClaI* fragment should shorten the size of this transcript. When pHW47 was used as the template to perform in vitro transcription assays, the size of this 310-nt transcript did not change (Fig. 4). These results indicate that the 310-nt transcript is not related to *rms* transcription but represents a distinct, separate transcript which initiates and terminates within the region upstream of the *rms*.

Demonstration of single promoter control in vivo of the rickettsial *rms* by RNase protection assays. To determine the in vivo initiation site and promoter location of the rickettsial *rms*, RNase protection assays were performed with not only the products of in vitro transcription but also total RNA isolated from rickettsia-infected host cells as the target RNA. Probe 1 (Fig. 2) covers a 480-bp upstream region that does not overlap the coding region for the mature product. If the *rms* promoter predicted from the in vitro transcription assays is correct, the 240-nt transcript should hybridize with this probe and provide a protected fragment of 71 nt in length. Any other transcript(s) initiated upstream and related to the *rms* should provide a longer protected (fully protected 480-nt) fragment(s). A 71-nt protected fragment was detected in the total RNA isolated from the rickettsia-infected mouse L929 cells but not from mock-infected cells (Fig. 5). These results demonstrate that the protected fragment was rickettsia specific, and under the experimental conditions in which the rickettsiae routinely grow, transcription of the rickettsial 16S rRNA was initiated from a single promoter. Also as shown in Fig. 5, only one dominant fragment (approximately 71 nt) was protected when transcripts generated in vitro from pHW44 were used as the target. No protected fragment could be found from in vitro transcripts generated from pHW47, which was used as a template for the 310-nt in vitro transcript.

Identification of the transcriptional start site of the rickettsial *rms*. On the basis of the information provided by in vitro transcription assays, RNase protection assays, and DNA sequencing, primer 2 (5'-GTACATTTTACTGTAC-3') was designed for mapping the precise site for the start of transcription. As shown in Fig. 6, one prominent band was revealed in RNA from the rickettsia-infected cells and from the product of the in vitro transcription assays. No band could be detected in the mock-infected cells. The initiation site of transcription was mapped to base A at position -117 upstream from the coding region for the mature product of the rickettsial *rms* (Fig. 3), which coincided well with the location defined by the in vitro transcription assays and RNase protection assays.

Control of the production of 16S rRNA in *R. prowazekii* in mouse L929 cells without supplemental methionine. One can ask whether, independently of mechanism, *R. prowazekii* controls 16S rRNA production in response to amino acid starvation. Amino acid starvation of *R. prowazekii* within mouse L929 cells was induced by removal of the essential amino acid methionine from the medium. The relative amounts of the newly synthesized precursors of 16S rRNA at various times after

FIG. 5. Identification of the specific transcript of the rickettsial *rrs* by RNase protection assays. Probe, undigested probe 1 (see Materials and Methods); Mock, RNA from mock-infected L929 cells; Rp, RNA from rickettsia-infected L929 cells; pHW44 and pHW47, in vitro transcripts produced by rickettsial RNAP with either pHW44 or pHW47 as the template, respectively. Arrow indicates the protected fragment.

starvation were determined by RNase protection assays and were corrected for rickettsial recovery by normalization of the band density of the probe 1-protected fragment (newly synthesized precursors of 16S rRNA) to the band density of the probe 2-protected fragment (total 16S rRNA). The numbers of rickettsiae per infected cell were significantly inhibited after 12 h of starvation. The amounts of newly synthesized precursors of 16S rRNA per rickettsia decreased after 6 h or more of starvation (Fig. 7). Furthermore, 12 h after the addition of methionine to the rickettsiae that had been starved for 12 h, the amounts of newly made 16S rRNA precursors per rickettsia increased (Fig. 7). The decrease in the amounts of the newly synthesized precursors of 16S rRNA per rickettsia could be caused by a decrease in 16S rRNA synthesis, an increased turnover of 16S rRNA precursors, or both.

The chemical half-lives of the newly synthesized precursors of 16S rRNA in *R. prowazekii* within mouse L929 cells with or without methionine starvation for 12 h were determined. Rifampin was used to block the initiation of transcription, and RNA was isolated at various times after the addition of rifampin. After RNase protection assays were performed, the amounts of the newly synthesized precursors of 16S rRNA and total 16S rRNA in each sample were determined. As shown in Fig. 8, the half-lives of the newly synthesized precursors of 16S rRNA were 13.7 min in the nonstarved rickettsiae and 13.1 min in the starved rickettsiae. No decay of the total 16S rRNA (which is predominantly mature 16S rRNA) was observed. Since the chemical half-life of the newly synthesized precursors of 16S rRNA in the starved rickettsiae did not differ significantly from that in the nonstarved rickettsiae, the decrease of 16S rRNA production per rickettsia observed was due to a decrease in 16S rRNA synthesis.

FIG. 6. Primer extension mapping of the site for the start of transcription in the rickettsial *rrs*. A, G, C, and T, sequencing ladder; M, RNA from mock-infected L929 cells; R, RNA from rickettsia-infected L929 cells; V, in vitro transcripts produced by rickettsial RNAP with pHW44 as the template. The sequence of the complementary strand is given. *, transcriptional start site at base A. Primer 2 (5'-GTACATTTTACTGTAC-3') was used for the extension.

DISCUSSION

Adaptation to alterations in the environment is a hallmark of bacterial growth. However, *R. prowazekii* can grow only in the cytoplasm of eukaryotic host cells, in a milieu which is very

FIG. 7. Effect of methionine starvation on the production of 16S rRNA in *R. prowazekii* within mouse L929 cells. pre16S, bands protected by probe 1, which detects the newly synthesized precursors of 16S rRNA; 16S, bands protected by probe 2, which detects total 16S rRNA; Time (h), hours after removal of methionine from the medium (after starvation for 12 h [12+], methionine [100 μ M] was added back to the medium for another 12 h); Density (%), densities of the pre16S bands in comparison with that of the pre16S band at 0 h. The densities of pre16S bands have been normalized to those of the 16S bands.

FIG. 8. Decay of pre16S and 16S rRNA in *R. prowazekii* within mouse L929 cells. (A) RNase protection assays of 16S rRNA stability. pre16S, bands protected by probe 1, which detects the newly synthesized precursors of 16S rRNA; 16S, bands protected by probe 2, which detects total 16S rRNA; +, RNA from nonstarved rickettsiae. -, RNA from rickettsiae starved for methionine for 12 h. (B) Logarithmic plots of RNA concentrations (not normalized) as a function of time.

different from that of free-living bacteria and, one would expect, more constant. Control of rRNA synthesis in *R. prowazekii* is of interest because, in free-living bacteria, rRNA transcription has enormous relevance to global control (8, 10, 22). In *E. coli*, redundant *rm* operons, two strong promoters per operon, and transcriptional regulatory sequences on each *rm* are believed to ensure that the production of rRNA is adequate for the bacteria to adapt to rapid growth rates (9–11). We demonstrated previously that in the genome of *R. prowazekii*, a slowly growing intracellular organism, there is only one 16S rRNA gene (16); however, surprisingly, the concentration of rRNA is very similar to that in more rapidly growing *E. coli* cells (17).

Within the region upstream of the rickettsial *rrs*, a promoter region responsible for the *rrs* expression was identified by RNase protection assays, in vitro transcription assays, and primer extension assays. This single promoter region controls the transcription of the rickettsial 16S rRNA under the conditions of normal rickettsial growth. This is strikingly different from *E. coli* and other free-living bacteria (8, 10, 22), in which dual or multiple promoters are usually present. As is the case for most rickettsial genes identified so far, the initiating nucleotide of the rickettsial *rrs* is a purine (base A). The rickettsial *rrs* has a promoter region located at 7 bp upstream of the

transcriptional start site and centered at 139 bp upstream of the coding region for the mature 16S rRNA product. The promoter region contained a -35 box (TTGACA), 17-bp spacer, and a -10 box (TATAAC), which suggests that the rickettsial promoter is similar (but not identical) to the consensus promoter of *E. coli* (a -10 box of TATAAT in *E. coli*). Yet, in contrast to what is known for *E. coli*, i.e., that *rm* promoters are among the most active (10), the promoter of the rickettsial *rrs* did not appear to be an unusually active promoter (data not shown) on the basis of the density of specific transcripts in vitro in comparison to *gltA* and *tlc* of *R. prowazekii*. A third promoter recognition region (A+T rich) is located in the upstream region of *E. coli* *rm* operons, which interacts directly with the α subunit of the *E. coli* RNAP (18). Since inspection of the upstream sequences of the rickettsial *rrs*, *gltA*, and *tlc* genes showed that all of them contain A+T-rich stretches, as expected for an A+T-rich genome (13), it is unknown whether the α subunit of the rickettsial RNAP plays a role in promoter recognition. Since multiple and highly active promoters are believed to ensure the high transcription rate of rRNA in free-living bacteria, especially at a high growth rate, the presence of a single promoter with unexceptional activity in the rickettsial *rrs* may reflect a low synthesis rate of the rRNA and ultimately the slow growth of the organism.

A sequence similar to that of the *E. coli* Fis-binding site (7) was found in the upstream region of the rickettsial *rrs* (Fig. 3 [nt 48 to 62]). Band shift assays with the *HindIII-XbaI* fragment from pHW45 containing this putative Fis-binding site established that this fragment could bind *E. coli* Fis protein. However, in experiments performed with purified *R. prowazekii* RNAP and a supercoiled plasmid template which contained the promoter region of the rickettsial *rrs*, the addition of *E. coli* Fis caused no significant enhancement of transcription. Indeed, one might question the necessity of this factor in *R. prowazekii*, a slowly growing intracellular bacterium, since the prime role of Fis in *E. coli* is to enable adaptation to a very high growth rate (15). However, it has other functions involved in recombination and site-specific DNA inversion (7).

As obligate intracellular bacteria, rickettsiae encounter challenges commonly faced by free-living bacteria, such as nutrient shortage, osmotic variation, and pH fluctuations, less frequently. In fact, the growth rates of *R. prowazekii* growing in a variety of cells (including avian, rodent, and human cells) do not vary substantially (25). However, the availability of nutrients does become limiting during the course of infection as the number of rickettsiae per host cell becomes massive. We show here that *R. prowazekii* regulates transcription of the rickettsial *rrs* in response to amino acid starvation. Such control might be akin to stringent control in other bacteria but appears kinetically different than the stringent response in *E. coli*. In rapidly growing *E. coli*, the synthesis of stable RNA stops almost immediately upon starvation (4), whereas the 16S rRNA synthesis in the slowly growing *R. prowazekii* gradually decreased after removal of methionine. In addition, there is as yet no evidence for the involvement of *rel*, *spoT*, or (p)ppGpp in *R. prowazekii*. Future studies to define a factor(s) related to this control in rickettsiae will provide valuable information about regulatory mechanisms in this obligate intracellular bacterium.

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