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Regulation of RraA, a Protein Inhibitor of RNase E-Mediated RNA Decay

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The recently discovered RraA protein acts as an inhibitor of the essential endoribonuclease RNase E, and we demonstrated that ectopic expression of RraA affects the abundance of more than 700 transcripts in *Escherichia coli* (K. Lee, X. Zhan, J. Gao, J. Qiu, Y. Feng, R. Meganathan, S. N. Cohen, and G. Georgiou, Cell 114:623–634, 2003). We show that *rraA* is expressed from its own promoter, P_{rraA}, located in the *menA-rraA* intergenic region. Primer extension and *lacZ* fusion analysis revealed that transcription from P_{rraA} is elevated upon entry into stationary phase in a σ^s-dependent manner. In addition, the stability of the *rraA* transcript is dependent on RNase E activity, suggesting the involvement of a feedback circuit in the regulation of the RraA level in *E. coli*.

RNase E is an essential protein that plays a crucial role in global mRNA metabolism as well as in the maturation of functional RNAs such as rRNAs, tRNAs, tmRNA, and small regulatory RNAs (3, 9, 18, 19, 21, 28). To date, RNase E homologs have been found in more than 50 eubacteria, archaebacteria, and plants (16). The cellular level and activity of RNase E are subjected to multiple environmental controls. At one level, RNase E synthesis is autoregulated by modulating the half-life of its own mRNA (12, 26). In addition, recent studies have revealed that 5′-monophosphorylated RNA serves as an allosteric activator of the endonuclease activity (13). Furthermore, the degradation of target RNAs by RNase E is found to be affected globally by endoribonuclease-binding proteins that control the decay and abundance of individual bacterial mRNAs in *trans* (8, 17).

RraA (*r*egulator of *r*ibonuclease *a*ctivity *A*), is an evolutionarily conserved 17.4-kDa protein with close homologs (>40% amino acid identity) in bacteria, archaea, proteobacteria, and plants. RraA binds to RNase E with an equilibrium dissociation constant (K_D) in the low-micromolar range and serves as a *trans*-acting modulator of the endonuclease activity of the enzyme (17). High-affinity binding requires the C-terminal half region of RNase E, which acts as a scaffold for the assembly of a large multiprotein complex called the degradosome (17, 36). RraA appears to interact only with the enzyme and not with RNA substrates (17). Gene chip analysis revealed that the action of RraA results in a dramatic change in the global abundance of mRNAs in *Escherichia coli*, affecting over 15% of all cellular transcripts. Importantly, the gene expression profile that is obtained upon overexpression of RraA is distinct from that obtained upon depletion of RNase E or through the action of RraB, a second *trans*-acting RNase E inhibitor of *E. coli* (8).

The *rraA* gene is located downstream of *menA*, which encodes a 1,4-dihydroxy-2-naphthoic acid octaprenyltransferase that catalyzes the prenylation of the redox mediator menaquinone (32). Transcription of *menA* appears to occur from a σ^{70}-dependent promoter. Earlier, Meganathan proposed that *rraA* (formerly designated *menG*) is transcribed from the *menA* promoter in a dicistronic mRNA (22). In this study we demonstrate that *rraA* is transcribed predominantly from its own promoter (P_{rraA}) located in the intergenic region between the *menA* and *rraA* genes. Transcription from P_{rraA} is σ^s dependent and is induced upon entry into stationary phase. Furthermore, we show that the synthesis of RraA is regulated at the post-transcriptional level by RNase E, suggesting the existence of a feedback regulatory circuit whereby induction of *rraA* transcription occurs in a σ^s-dependent manner and results in inhibition of RNase E activity, in turn decreasing the degradation rate of the *rraA* transcript.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phage vectors. The strains, plasmid, and phage vectors used in this study are listed in Table 1. The *rpoS*::*kan* mutation from strain ZK1000 was introduced into various strains by P1 transduction as described by Miller (24). The transduction of the *rpoS* disruption was confirmed by streaking single kanamycin-resistant transductants onto Luria-Bertani broth (LB)-kanamycin agar plates and testing the ability of catalase to hydrolyze hydrogen peroxide.

Growth conditions. LB and M9 minimal medium (23) containing thiamine (50 μg/ml), 0.4% (wt/vol) glucose, and 0.2% (wt/vol) casein were supplemented with antibiotics, as required (50 μg/ml ampicillin, 25 μg/ml kanamycin, or 15 μg/ml tetracycline). Unless otherwise stated, cells were grown in LB medium under aeration at 37°C, and the growth was monitored by measuring the absorbance at 600 nm.

RNA methods. For reverse transcriptase-PCR (RT-PCR) analysis, total RNA was isolated with the RNeasy kit (QIAGEN, Valencia, CA) and treated with RNase-free DNase (Ambion, Austin, TX). Fifty ng of total RNA was subjected to RT-PCR analysis using the One Step RNA PCR kit (TaKaRa, New York, NY).

Northern blot assays were performed using total RNA isolated from *E. coli* JCB570 grown as described above. Samples were collected at 1-hour intervals throughout the exponential and stationary phases. Five μg of total RNA per lane

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TABLE 1. Strains, plasmids, and phage vectors

Strain, plasmid, or phage vector	Description	Reference or source
Strains		
JCB570	MC1000; *phoR zih12*::Tn*10*	1
ZK126	W3110; Δ*lacU16 tna-2*	4
ZK1000	ZK126; *rpoS*::*kan*	4
EC-O	*thi-1 relA1* Δ(*pro-lac*)*X113*[*del* = *DE5*] *supE44*/*F42-114*(FTs) *lac*	Lab collection
DY330	W3110; Δ*lacU169 gal 490* λcI857 Δ(*cro-bioA*)	39
MZ001	EC-O; λ p0-*lacZ*	This study
MZ002	EC-O; λ (nt −1076 to −1)-*lacZ*	This study
MZ003	EC-O; λ (nt −92 to −1)-*lacZ*	This study
MZ004	EC-O; λ (nt −1076 to −93)-*lacZ*	This study
MZ013	MZ003; *rpoS*::*kan*	This study
MZ014	MZ004; *rpoS*::*kan*	This study
MZ100	JCB570; *rpoS*::*kan*	This study
CH1827	MC1061; *zce-726*::Tn*10*	27
CH1828	CH1827; *rne-1*	25
Plasmids		
pSP417	pBR ori Amp^r	29
pMZ002	pSP417 (nt −1076 to −1)-*lacZ*	This study
pMZ003	pSP417 (nt −92 to −1)-*lacZ*	This study
pMZ004	pSP417 (nt −1076 to −93)-*lacZ*	This study
Phage vectors		
λRS45	*bla′-lacZ*$_{sc}$ *imm21 ind*$^+$	33
λRS74	*placUV5-lacZ*$^+$ *imm21 ind*$^+$	33
λMZ1	Same as λRS74 but containing the p0-*lacZ* fusion	This study
λMZ2	Same as λRS45 but containing the (nt −1076 to −1)-*lacZ* fusion	This study
λMZ3	Same as λRS45 but containing the (nt −92 to −1)-*lacZ* fusion	This study
λMZ4	Same as λRS45 but containing the (nt −1076 to −93)-*lacZ* fusion	This study

was loaded onto a denaturing gel containing formaldehyde and then transferred to a positively charged nylon membrane (Hybond N$^+$; Amersham, United Kingdom). The AlkPhos direct nucleic acid labeling and detection system (Amersham, United Kingdom) was used for probe synthesis. Hybridization, washing of the membranes, and detection of signals were carried out according the manufacturer's protocol.

For primer extension, a 5′-^{32}P-labeled oligonucleotide (5′-GCGGTTCCACG ACGTTAACATCTTCTTGA-3′ or 5′-CCGTCCGCCAAAGTTGGAGAACA GC-3′) was used as a primer for the RT reaction with 5 μg of total RNA (SuperScript III RNase H$^-$ reverse transcriptase; Invitrogen, Carlsbad, CA). The primer extension products were separated on 6% polyacrylamide-7 M urea gels. The dideoxy-DNA sequence ladder from the same primer was prepared using the *fmol* DNA Cycle Sequencing System (Promega, Madison, WI).

For RNase protection assays, *E. coli* CH1827 (MC1061; *zce-726*::Tn*10*) or CH1828 (CH1827; *rne-1*) was grown in LB at 30°C to an A_{600} of 0.4 and then half of the culture was transferred to 43.5°C and incubated for an additional 20 min. After the addition of rifampin (500 μg/ml), aliquots were withdrawn every 45 s, immediately chilled, and stored in liquid nitrogen. Total RNA was prepared from the frozen samples by using the RNeasy kit (QIAGEN, Valencia, CA), and RNase protection assays were performed using the RPA III kit (Ambion, Austin, TX) with the in vitro transcript from the complementary strand of the *rraA* gene (nucleotides [nt] +13 to +318 of *rraA*) as the probe. The band intensity was quantified using ImageQuant software.

Construction of β-galactosidase fusions. We used PCR amplification to generate DNA fragments containing different regions upstream of *rraA*, i.e., nt −1076 to −1 of the *rraA* upstream region, which includes the P*menA*, *menA* coding sequence, and *menA-rraA* intergenic region; nt −1076 to −93, which includes the P*menA* and *menA* coding sequence; and nt −92 to −1 of the *menA-rraA* intergenic region, and cloned each fragment upstream of the *lacZ* gene in a multicopy transcriptional fusion vector, pSP417 (29). So we generated plasmids pMZ002 ([nt −1076 to −1]-*lacZ*), pMZ003 ([nt −92 to −1]-*lacZ*), and pMZ004 ([nt −1076 to −93]-*lacZ*); pMZ001 was the negative-control vector. The *lacZ* fusions in pMZ002, pMZ003, and pMZ004 were transferred onto the chromosome using the transducing lambda phage system (33). The fusions were transferred into λRS45, whereas the negative-control fusion in pMZ001 was transferred into λRS74 via a double recombination event. Plaques containing the recombinant lambda phages were isolated based on their blue plaque phenotype. The recombinant lambda phages were used to lysogenize EC-O [Δ(*pro-lacZ*)] and generated the following strains: MZ001 (EC-O; λ p0-*lacZ*), with a chromosomal promoterless *lacZ* gene; MZ002 (EC-O; λ [nt −1076 to −1]-*lacZ*), harboring nt −1076 to −1 of the *rraA* upstream region fused to *lacZ*; MZ003 (EC-O; λ [nt −92 to −1]-*lacZ*), harboring nt −92 to −1 of the *menA-rraA* intergenic region fused to *lacZ*; and MZ004 (EC-O; λ [nt −1076 to −93]-*lacZ*), harboring nt −1076 to −93 of the *rraA* upstream region fused to *lacZ*. All lysogens were tested for monolysogenization by PCR (30).

β-Galactosidase assays. Cultures grown with aeration at 37°C, in rich or M9 medium overnight, were subcultured into the fresh medium. Samples were collected at exponential and stationary phases. The samples were collected at 4°C and resuspended in an appropriate volume of ice-cold Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM M KCl, 1 mM $MgSO_4$; pH 7.0) (23) to give an A_{600} in the range from 0.6 to 0.9. β-Galactosidase activities were determined from at least three independent experiments, as previously described (24).

Western immunoblotting. The cells were harvested by centrifugation, resuspended in phosphate-buffered saline, and lysed by passage through a French press (2,000 lb/in^2). The lysate was centrifuged for 15 min at 4°C to remove cell debris, and the protein concentration in the supernatant was determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA). One μg of total protein was separated by sodium dodecyl sulfate-12% polyacrylamide gel electrophoresis. Western blot assays were performed by standard methods using monoclonal anti-FLAG-M2-peroxidase (horseradish peroxidase) antibody conjugate (Sigma, St. Louis, MO). Signal intensities were measured, quantified by molecular analysis software (Quantity One; Bio-Rad), and displayed below the blot image.

RESULTS AND DISCUSSION

Identification of the P$_{rraA}$ promoter. Meganathan had proposed that *rraA* is transcribed as a dicistronic mRNA from the

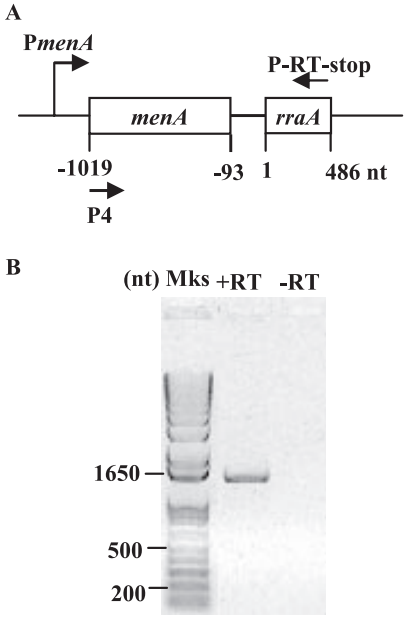


FIG. 1. RT-PCR analysis. A. The annealing positions of the reverse primer (P-RT-stop) and forward primer (P4) used in the RT-PCR analysis of the *menA-rraA* region. B. RT-PCR analysis of *rraA* transcript using the primers P4 and P-RT-stop. −RT, the negative control containing the same amounts of RNA, primers, and *Taq* polymerase but no reverse transcriptase. Lane Mks, molecular size markers.

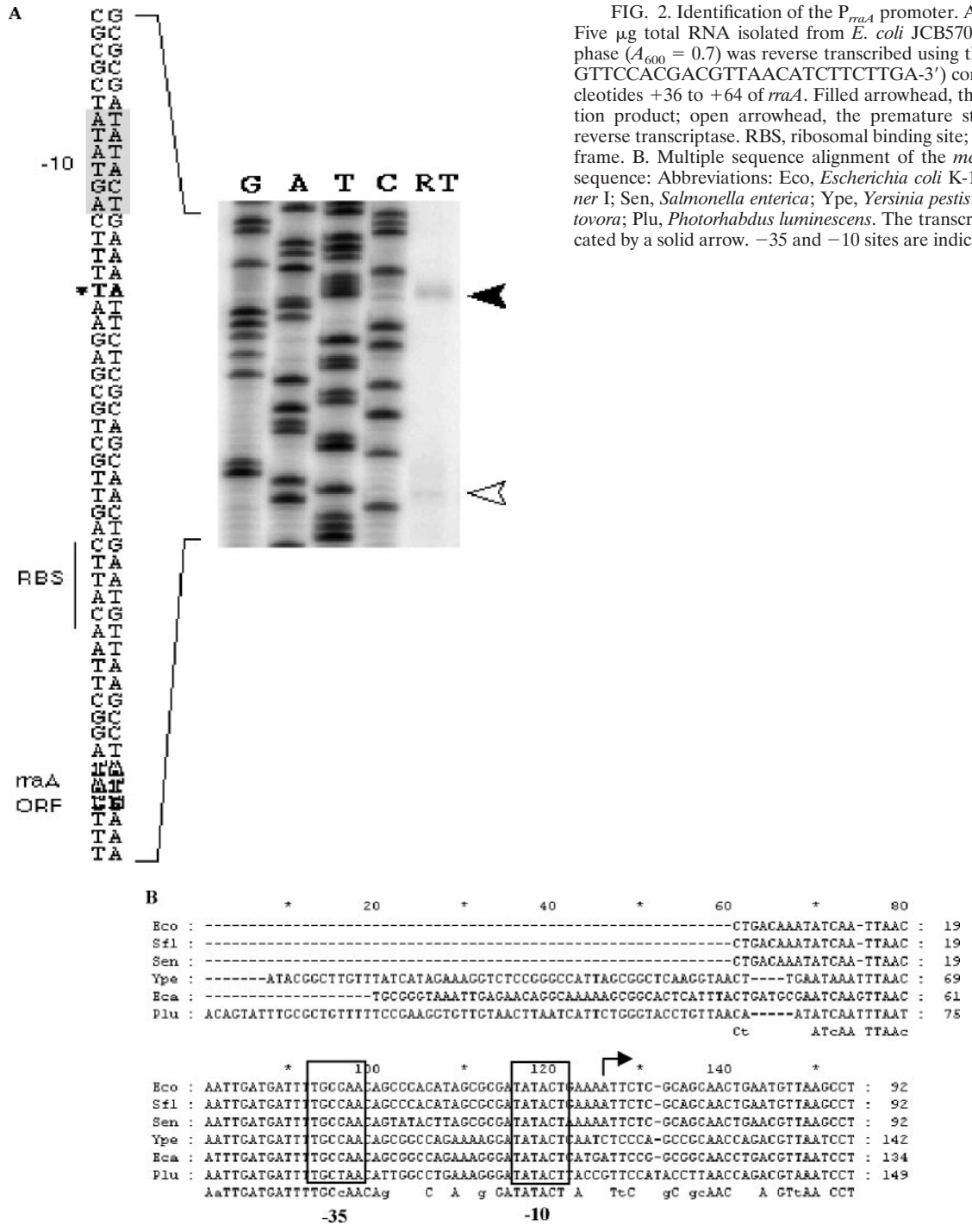


FIG. 2. Identification of the P_{rraA} promoter. A. Primer extension. Five μg total RNA isolated from *E. coli* JCB570 cells grown to log phase (A_{600} = 0.7) was reverse transcribed using the primer (5′-GCG GTTCCACGACGTTAACATCTTCTTGA-3′) complementary to nucleotides +36 to +64 of *rraA*. Filled arrowhead, the reverse transcription product; open arrowhead, the premature stop product of the reverse transcriptase. RBS, ribosomal binding site; ORF, open reading frame. B. Multiple sequence alignment of the *menA-rraA* intergenic sequence: Abbreviations: Eco, *Escherichia coli* K-12; Sfl, *Shigella flexner* I; Sen, *Salmonella enterica*; Ype, *Yersinia pestis*; Eca, *Erwinia carotovora*; Plu, *Photorhabdus luminescens*. The transcript start site is indicated by a solid arrow. −35 and −10 sites are indicated by open boxes.

P*menA* promoter (22). The transcription initiation site for this putative dicistronic transcript was identified at 57 bp upstream of the *menA* translation start site (34). Consistent with this hypothesis, a *menA-rraA* transcript of the expected size was detected by RT-PCR analysis (Fig. 1), suggesting that *rraA* is transcribed together with the preceding *menA* as a dicistronic mRNA. However, Northern blot analysis with two different *rraA*-specific probes revealed that the predominant RNA in samples in either exponential or stationary phase corresponds to a shorter species, ca. 550 bases in length (data not shown). Primer extension of RNA isolated from *E. coli* JCB570 using the 5′-^{32}P-labeled oligonucleotide probe ^{32}P-GCGGTTCCAC GACGTTAACATCTTCTTGA revealed an *rraA* transcript that is initiated at an A residue 28 nt upstream from the ATG codon (Fig. 2A). In addition to this major transcript, the data in Fig. 2A also showed a faint band at the position of the initiation codon of *rraA* (indicated by an open arrowhead). However, this band was not detected in a primer extension

assay using an alternative primer (^{32}P-CCGTCCGCCAAAGT TGGAGAACAGC) that anneals to a different location within the *rraA* RNA (located 23 bp upstream from the first primer), suggesting that the minor band likely corresponds to a premature stop product of the reverse transcriptase.

DNA sequence analysis of the *menA-rraA* intergenic region suggested the existence of a putative promoter (P_{rraA}) (Fig. 2B). *rraA* and *menA* are separated by a 92-bp intergenic region that shows a significant degree of conservation among closely related bacteria such as *Shigella flexneri* (92/92 bp, 100% identity in the intergenic region) and *Salmonella enterica* (86/92 bp, 93% identity in the intergenic region). A lower degree of sequence identity was observed for phylogenetically more distant bacteria, *Yersinia pestis*, *Erwinia carotovora*, and *Photorhabdus luminescens*. Analysis using GENETYX-MAC 11.2.5 identified regions that match the σ^{70} consensus −35 and −10 sequences, centered at 32 and 8 nucleotides, respectively, upstream of the transcription initiation site (Fig. 2B). Multiple sequence alignment, using CLUSTAL X 1.8, among the bacterial species which have close homologs (>80%) of *E. coli* K-12 *rraA* revealed that the sequence of the P_{rraA} promoter is conserved among gammaproteobacteria. As shown in Fig. 2B, the −35 and −10 regions are identical, except for *Photorhabdus luminescens*, whose −35 region differs from that of *E. coli* K-12 by 1 bp.

Genetic analysis of expression of P_{rraA} using *lacZ* transcriptional fusions. We used PCR amplification to generate DNA fragments containing different regions upstream of *rraA* extending up to, and including, the *menA* promoter, as shown in Fig. 3A, and cloned each fragment upstream of the *lacZ* gene in a multicopy transcriptional fusion vector, pSP417 (29). In this way, we generated plasmids pMZ002 ([nt −1076 to −1]-*lacZ*), pMZ003 ([nt −92 to −1]-*lacZ*), and pMZ004 ([nt −1076 to −93]-*lacZ*); pMZ001 was the negative-control vector.

To rule out the possibility that differences in the β-galactosidase activity expressed from the above transcriptional fusions might be partially due to plasmid copy number effects, we then made single chromosomal copy isolates of each construct using the transducing lambda phage system (33). The *lacZ* fusions in pMZ001, pMZ002, pMZ003, and pMZ004 were transferred into either λRS74 or λRS45 via a double recombination event to generate λMZ1, λMZ2, λMZ3, and λMZ4, respectively. Subsequently, *E. coli* EC-O was lysogenized with the recombinant phages to generate strains carrying a single copy of each promoter-*lacZ* fusion. As shown in Fig. 3B, strain MZ003 (EC-O; λ [nt −92 to −1]-*lacZ*), with only the *menA-rraA* intergenic region fused to *lacZ*, showed levels of β-galactosidase activity identical to those observed in MZ002 (EC-O; λ [nt −1076 to −1]-*lacZ*), which contains a fusion to the *rraA* upstream region, including the P*menA* and *menA* coding sequence. In contrast, MZ004 (EC-O; λ [nt −1076 to −93]-*lacZ*), containing a fusion to nt −1076 to −93 of the *rraA* upstream region that lacked the *menA-rraA* intergenic region, showed 67% lower activity.

***rraA* expression in stationary phase.** Earlier microarray studies had shown that the *rraA* mRNA level is increased upon entrance to stationary phase (https://asap.ahabs.wisc.edu/annotation/php/ASAP1.htm). The abundance of the RraA protein in exponential- and stationary-phase cells was examined by Western blotting using strain DY330-*rraASPA* (5, 39), in which sequential peptide affinity (SPA) tags are fused to the C terminus of the *rraA* open reading frame within the chromosome. We found that the level of RraA protein increased by about twofold in stationary phase (Fig. 4A). Many genes that are upregulated in stationary phase are part of the *rpoS* regulon. The *E. coli rpoS* regulon comprises more than 100 genes that are transcribed by the sigma factor σ^s (or σ^{38}, RpoS) and are upregulated in stationary phase or under certain stress conditions such as hyperosmolarity, low pH, and oxidative and temperature stresses and function largely in stress response, carbon metabolism, and cell envelope biogenesis (10). In order to determine if *rraA* transcription is dependent on the growth phase, we examined the *rraA* promoter activity in exponential- or stationary-phase cells grown in LB medium at 37°C. Primer extension analysis of RNA from stationary-phase cells revealed a marked increase in the *rraA* transcript in stationary-phase cells (Fig. 4B). In addition to the *rraA* transcript, several minor bands were also evident in samples from stationary-phase cells. These correspond to premature termination or misannealing products since neither could they be detected consistently nor were they detected in a primer extension assay using a different primer. No increase in the level of *rraA* transcript in stationary-

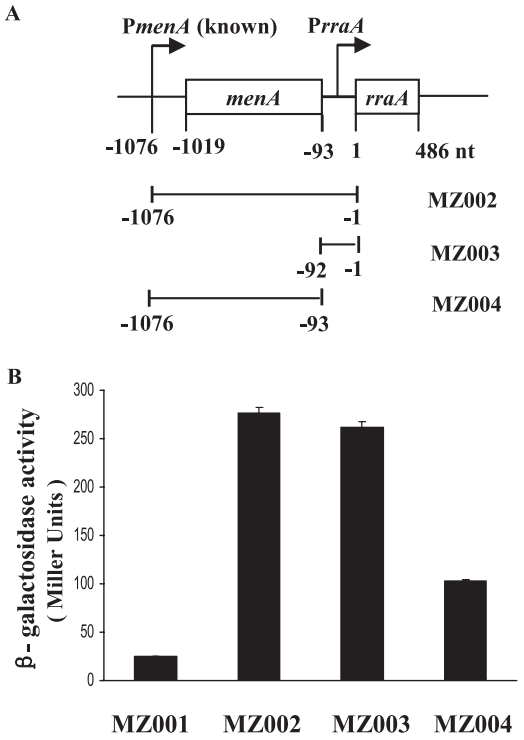
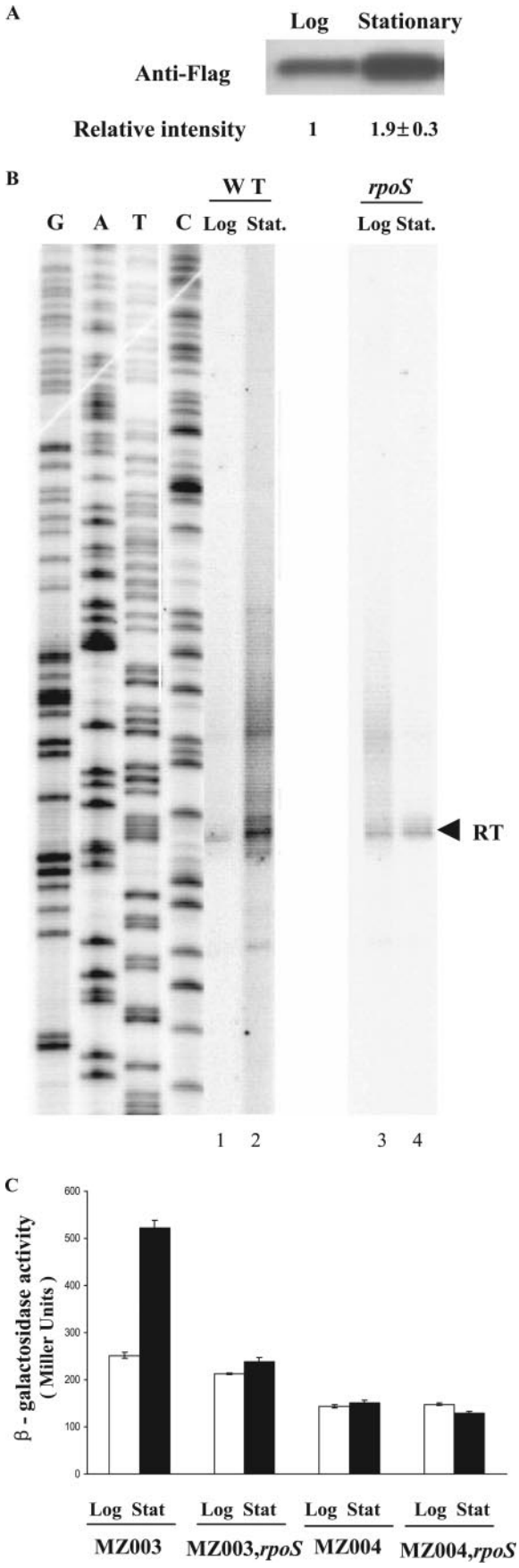


FIG. 3. *lacZ* transcriptional fusions. A. Schematic of the transcriptional *rraA-lacZ* fusions used in this study. B. β-Galactosidase activities in MZ001, MZ002, MZ003, and MZ004 cells. MZ001 (EC-O; λ p0-*lacZ*) has a chromosomal promoterless *lacZ* gene; MZ002 (EC-O; λ [nt −1076 to −1]-*lacZ*) has nt −1076 to −1 of the *rraA* upstream region fused to *lacZ*; MZ003 (EC-O; λ [nt −92 to −1]-*lacZ*) has nt −92 to −1 of the *menA-rraA* intergenic region fused to *lacZ*; MZ004 (EC-O; λ [nt −1076 to −93]-*lacZ*) has nt −1076 to −93 of the *rraA* upstream region fused to *lacZ*. Cells were grown in LB under aeration at 37°C and harvested in log phase (A_{600} = 0.5 to 0.8). Samples were normalized by optical density, and enzymatic activities were measured in Miller units. The data presented are averages of at least three independent determinations, and error bars correspond to the standard deviations.



phase cultures was observed in the *rpoS* mutant strain MZ100 (JCB570; *rpoS*::*kan*). Collectively, these results indicate that transcription from the P_{rraA} promoter increases in stationary phase and this effect is dependent on the transcription factor σ^s.

The effect of *rpoS* on the transcriptional activity of the P_{rraA} promoter was also evaluated using *lacZ* fusions transcribed from the P_{rraA} promoter. Specifically, β-galactosidase activity was determined in strains MZ003, MZ004, MZ013 (MZ003; *rpoS*::*kan*), and MZ014 (MZ004; *rpoS*::*kan*) grown to either exponential or stationary phases. As shown in Fig. 4C, in strain MZ003, the β-galactosidase activity increased more than twofold in stationary-phase cultures ($A_{600} \approx 2.5$) compared with log-phase cells ($A_{600} \approx 0.6$). In the isogenic *rpoS* mutant MZ013, the β-galactosidase activity was comparable to the level observed in parental strain MZ003 grown in exponential phase, but the induction of *lacZ* expression in stationary phase was abolished. As a control, the β-galactosidase activity of MZ004 and MZ014 cells where *lacZ* is transcribed from the upstream *menA* promoter was neither growth phase dependent nor affected by *rpoS*. These results clearly suggest that σ^s is responsible for the increased transcription from P_{rraA} upon entrance into stationary phase. Recently, using microrarray analysis Hengge-Aronis's group identified over 400 genes which are positively controlled by σ^s in *E. coli*. While *rraA* was not recognized as a σ^s-dependent gene in this study, we note that 33 out of the 87 genes which had been experimentally shown to be σ^s controlled in earlier studies also failed to be detected in the microarray experiments (37).

Many σ^s promoters contain an extended −10 region, KCT AYRCTTAA (nucleotides −14 to −4; K stands for T or G, Y stands for T or C, and R stands for A or G) (37). Particularly, C at −13 has been shown to interact directly with lysine 173 in the 2.5 region of σ^s (2, 7, 31, 37). Nevertheless, despite its

FIG. 4. *rraA* expression in stationary phase. A. Western immunoblot analysis of RraA level in exponential and stationary phases. Strain DY330-*rraA*SPA, bearing a sequential peptide affinity (SPA) tag at the C terminus of the *rraA* open reading frame, was cultured in LB medium at 32°C and harvested during exponential growth ($A_{600} = 0.4$) and stationary phase ($A_{600} = 3.5$). RraA is expressed at an endogenous level from its chromosomal promoter and detected by the highly specific anti-FLAG antibody. Blots were replicated from three independent protein preparations. Replicate measurements were made on the same membrane to determine the reproducibility of the analysis. The intensity of the signal was observed to be linear by using a dilution series of total protein. The data are the means and errors of three separate blots. B. Primer extension analysis of the transcription from P_{rraA} in growth phases. Total RNA was prepared from isogenic strains JCB570 (WT, wild type) and MZ100 (JCB570; *rpoS*::*kan*) grown to log phase ($A_{600} = 0.6$) and stationary phase ($A_{600} = 2.5$). Equivalent amounts of RNA (5 μg) were reverse transcribed using the primer (5′-GCGGTTCCACGACGTTAACATCTTCTTGA-3′) complementary to nucleotides +36 to +64 of *rraA*. The position of the reverse transcription product is shown by the arrowhead. C. Effect of σ^s level on the transcriptional activity of the various P_{rraA}-*lacZ* fusions in growth phases. Strains MZ003 (EC-O; λ [nt −92 to −1]-*lacZ*), MZ004 (EC-O; λ [nt −1076 to −93]-*lacZ*), MZ013 (MZ003; *rpoS*::*kan*), and MZ014 (MZ004; *rpoS*::*kan*) were grown in log ($A_{600} = 0.6$) and stationary ($A_{600} = 2.5$) phases in M9 medium supplemented with 0.2% casein with aeration, at 37°C. Samples were normalized by optical density, and enzymatic activities were measured in Miller units. The data presented are averages of at least three independent determinations, and error bars correspond to the standard deviations.

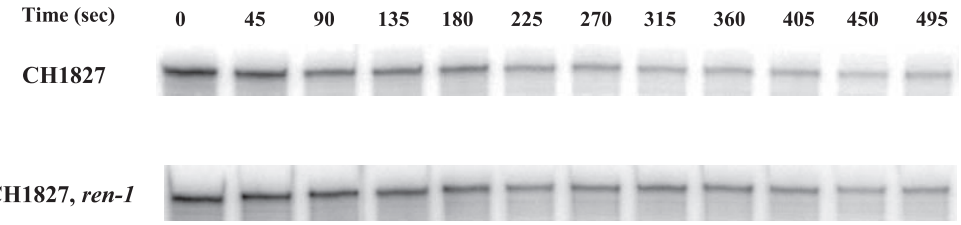


FIG. 5. Degradation profile of the *rraA* mRNA in wild-type and *rne-1* strains. RNase protection assays and total RNA isolation were done as described in Materials and Methods on strains CH1827 (MC1061; *zce-726*::Tn*10*) and CH1828 (CH1827; *rne-1*). Time points in seconds were sampled after rifampin addition. Equivalent amounts of RNA (5 μg) were used in RNA protection assays and loaded into each lane of a 6% polyacrylamide-7 M urea gel. The band intensity was quantified using ImageQuant software. Degradation rates were determined by fitting the linear regression model and represent the averages of two independent determinations.

importance, C at −13 is not absolutely essential for more efficient promoter utilization by σ^s than by σ^{70}, as shown with mutant variants of the *aidB* promoter (11, 15). The extended −10 motif is found only on the genes which are σ^s dependent under all three growth and stress conditions (i.e., transition into stationary-phase growth, hyperosmotic shift, and low pH). They account for less than 30% of the total genes which are positively controlled by *rpoS* and were named the "core set" of σ^s-dependent genes (37). Genes that display σ^s dependence only under certain conditions do not contain this extended −10 sequence (37). In P_{rraA}, the extended −10 region (GATAT ACT) contains an A rather than a C at −13, suggesting that additional factors may be involved in sigma factor selectivity. For example, the well-characterized σ^s-controlled gene *csiD*, which is mainly induced by carbon starvation, contains an A at −13 (−10 sequence, GATATTTT) (20, 38) and requires cyclic AMP (cAMP)-cAMP receptor protein for sigma factor selectivity.

We could not identify sequences resembling either the distal or the proximal UP element, which has been proposed to enhance σ^s selectivity (35). The absence of an apparent UP element is not surprising, as such sequences are absent in more than 50% of the known σ^s-dependent promoters (35). It should be noted that extensive earlier studies have revealed that σ^s promoter selectivity is affected by a number of factors including DNA supercoiling, topology, and the action of additional protein regulators (6, 14, 31, 37).

The decay of the *rraA* transcript is dependent on RNase E. Since the function of RraA is to modulate the endonuclease activity of RNase E, it was of interest to examine whether RNase E plays a role in the decay of the *rraA* transcript. Because RNase E is an essential protein, the stability of the *rraA* mRNA was examined in *E. coli* CH1828, which contains the temperature-sensitive *rne-1* allele. Cells were grown at 30°C in LB medium under aerobic conditions to mid-log phase, followed by a temperature upshift to 43.5°C for 20 min. Rifampin was added to inhibit transcription, samples were withdrawn at different times, and the level of *rraA* was determined by RNase protection assays (Fig. 5). In the parental strain CH1827, the half-life of *rraA*, determined by fitting the linear regression model, was virtually identical at 30°C and at 43.5°C ($t_{1/2}$ = 2.4 ± 0.3 and 2.9 ± 0.2 min, respectively). However, incubation of the *rne-1* mutant strain at the nonpermissive temperature resulted in a significant (twofold) stabilization of the *rraA* transcript, whose $t_{1/2}$ increased to 6.1 ± 1.1 min. Since RraA acts as an inhibitor of RNase E activity, the finding that the processing of the *rraA* transcript is RNase E dependent suggests the existence of a feedback mechanism: induction of *rraA* synthesis, possibly by environmental triggers that stimulate σ^s-dependent transcription, would be expected to result in higher accumulation of RraA protein, in turn inhibiting RNase E activity and thus leading to stabilization of the *rraA* transcript. The net result would be a further increase in the level of RraA protein and greater inhibition of RNase E activity.

The microarray data of Lee et al. (17) revealed that either overexpression or deletion of *rraA* affect the steady-state abundance of a number of transcripts from σ^s-dependent genes. It is noteworthy that the *rpoS* transcript and its regulators *rseABC* have been shown to be stabilized by either RraA overexpression or RNase E depletion (17). Accordingly, a possible biological role for the σ^s-dependent P_{rraA} activity in early stationary phase may be to provide a means for the protection of σ^s-dependent transcripts from the decay catalyzed by RNase E.

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