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The *trmA* Promoter Has Regulatory Features and Sequence Elements in Common with the rRNA P1 Promoter Family of *Escherichia coli*

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The tRNA(m^5U54)methyltransferase, whose structural gene is designated *trmA*, catalyzes the formation of 5-methyluridine in position 54 of all tRNA species in *Escherichia coli*. The synthesis of this enzyme has previously been shown to be both growth rate dependent and stringently regulated, suggesting regulatory features similar to those of rRNA. We have determined the complete nucleotide sequence of the *trmA* operon in *E. coli* and the sequence of the *trmA* promoter region in *Salmonella typhimurium* and also analyzed the transcriptional regulation of the gene. The *trmA* and the *btuB* (encoding the vitamin B_{12} outer membrane receptor protein) promoters are divergent promoters separated by 102 bp between the transcriptional start sites. The *trmA* promoters of both *E. coli* and *S. typhimurium* share promoter elements with the rRNA P1 promoter. The sequence downstream from the −10 region of the *trmA* promoter is homologous to the discriminatory region found in stringently regulated promoters. Next to and upstream from the −10 region is a sequence, TCCC, in the *trmA* promoter that is present in all of the seven rRNA P1 promoters and in some tRNA promoters but not in any other σ^{70} promoter. However, a similar motif is also found in promoters transcribed by the heat shock sigma factor σ^{32}. The *trmA* gene is transcribed as a monocistronic operon, and the 3′ end of the transcript is shown to be located downstream from a dyad symmetry region not followed by a poly(U) stretch. Using a *trmA-cat* operon fusion, we show that the growth rate-dependent regulation of *trmA* resembles that of rRNA and operates at the level of transcription.

tRNA is the most heavily modified nucleic acid in the bacterial cell. It has been estimated that at least 45 different tRNA-modifying enzymes are present in *Escherichia coli* (4, 5). The *trmA* gene encodes the enzyme tRNA(m^5U54)methyltransferase (EC 2.1.1.35), which catalyzes the formation of 5-methyluridine (m^5U, ribothymidine) at position 54 in all tRNAs of *E. coli* (36). The *trmA* gene is located at min 89 between the *oxyR* and the *btuB* genes on the *E. coli* genetic map (Fig. 1) (2). The direction of transcription is counterclockwise, and transcription studies in vitro have identified a promoter region responsible for *trmA*-specific transcription (28).

The *trmA* gene is expressed at a low level, resulting in approximately 200 tRNA(m^5U54)methyltransferase molecules per cell when grown in glucose minimal medium (37). The *trmA* gene shows basic regulatory features similar to those of rRNA genes under many different physiological conditions and situations, such as (i) growth, (ii) during nutritional shift-down and shift-up, (iii) during amino acid limitation in a *relA*$^+$/*relA1* background, (iv) in a *fusA* (temperature-sensitive elongation factor G) mutant, which at nonpermissive temperatures overproduces rRNA, and (v) in a *fusB* (gene product not identified) mutant, which at nonpermissive temperatures represses the synthesis of rRNA (34, 35). Thus, the regulation of expression of the *trmA* gene appears similar to that of rRNA genes. Unlike rRNA genes, the *trmA* gene responds to gene dose as would be expected according to the ribosome feedback model (33, 36).

In order to analyze the regulatory features of the *trmA* operon, we have determined the nucleotide sequence of the *trmA* gene in *E. coli* and the nucleotide sequence covering the promoter region of *trmA* from *Salmonella typhimurium*. The *trmA* transcripts in vivo were characterized by mapping of the 3′ and 5′ ends, and we also show that the growth rate-dependent regulation operates at the level of transcription. An analysis of the *trmA* promoter sequences from both *E. coli* and *S. typhimurium* revealed a highly conserved element so far found only in rRNA P1 promoters and in some tRNA promoters but not in any other σ^{70} promoter.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used are listed in Table 1. LB medium was used (39) for routine growth of bacteria. Physiological experiments were carried out by using MOPS (morpholinepropanesulfonic acid)-minimal medium (31) supplemented with 0.4% glycerol or glucose. For rich medium, it was also supplemented with amino acids, vitamins, and nucleosides. All supplements were provided at the concentrations recommended by Davis et al. (11). The growth rate was determined by the rate of increase in cell mass between optical densities at 420 nm of 0.1 and 1.0. Growth rates are expressed as the specific growth rate constant ($k = \ln 2/\mu$). Antibiotics included (in micrograms per milliliter) ampicillin (50), carbenicillin (50), chloramphenicol (10), and kanamycin (50) as needed.

Construction of plasmids for DNA sequencing. DNA upstream of the previously identified *trmA* promoter (28) was cloned as follows. Plasmid pGP1843, carrying the *trmA*$^+$ gene together with *argH*$^+$, was obtained through partial digestion of λd*arg*13 with *Hin*dIII following ligation into

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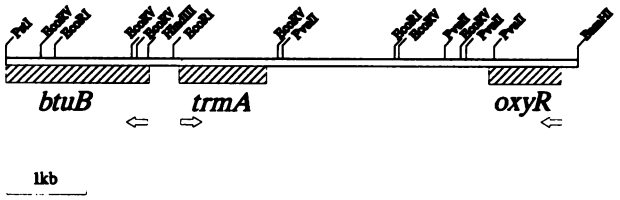

FIG. 1. Genetic and physical map of the region surrounding the *trmA* gene at min 89 of the *E. coli* chromosome. Arrows indicate directions of transcription. The location and physical maps of the structural genes *btuB* and *oxyR* are based on those of Heller and Kadner (21) and Tao et al. (44).

*Hin*dIII-digested plasmid pBR322. The ligation mix was transformed into strain GB802 (*argH1 trmA5 recA1*). As the *trmA* gene is located 6 kb from *argH*$^+$ on the *E. coli* chromosomal map, arginine prototrophs were selected. Arg$^+$ transformants carrying a plasmid containing an insert, which included the 2.8-kb *Eco*RI fragment previously shown to contain the *trmA*$^+$ gene (36), were chosen. Partial digestion of plasmid pGP1843 with *Hin*dIII and *Eco*RI resulted in plasmid pGP1509, which carries the 2.8-kb *Eco*RI fragment encoding tRNA(m^{5}U54)methyltransferase (36) as well as a 311-bp *Hin*dIII-*Eco*RI fragment upstream of the previously mapped (28) promoter region of the *trmA* gene. The nucleotide sequence of the 1,684-bp *Hin*dIII-*Pvu*II fragment of *E. coli* carrying the *trmA*$^+$ gene was determined from various fragments of plasmid pGP100 or pGP1509 (see Fig. 2).

To clone the *trmA*$^+$ gene from *S. typhimurium*, a genomic library was constructed from partially digested (with *Sau*3AI) chromosomal DNA of *S. typhimurium* GT344, which was ligated into the *Bam*HI site of the low-copy-number kanamycin resistance plasmid pLG339 essentially as described by Sambrook et al. (39). Strain GB1-5-797 (*trmA argH*) was transformed with the genomic library, and transformants were selected for Arg$^+$ and Km^r. Cell extracts from Arg$^+$ Km^r transformants were assayed for tRNA(m^{5}U54) methyltransferase activity in vitro (34). Plasmids from cells that could methylate tRNA also hybridized to a *trmA* probe spanning the region from *Eco*RI (position 312) to *Cla*I (position 887) in the *E. coli trmA* gene (data not shown). The probe was labeled with an oligolabeling kit (Pharmacia-LKB Biotechnology, Sollentuna, Sweden) as described by the manufacturer.

One of the plasmids, pUST105, with an insert of 12 kb, was chosen, and the *trmA* promoter was further subcloned on a 1.2-kb *Cla*I-*Cla*I fragment into pBluescript SK (Stratagene, La Jolla, Calif.), resulting in plasmid pUST106, which was used to sequence the promoter region in both orientations.

DNA sequencing. DNA fragments were cloned into different M13 derivatives and used as templates for DNA sequence analysis essentially as described by Messing and Vieira (30). DNA sequence analysis was performed by either dideoxy-chain termination sequencing as described by Sanger et al. (40) on M13 derivatives or chemical sequencing as described by Maxam and Gilbert (29). Plasmids were sequenced according to the Sequenase (United States Biochemical) manual. Sequence analysis was performed by utilizing the computer program GENEUS described by Harr et al. (20).

S1 and T2 nuclease mapping of transcriptional start points and endpoints. As a template for the synthesis of antisense RNA, we used plasmid pCG14 carrying the *trmA* promoter region from *Bss*HII (position 526) to *Eco*RI (position 312)

TABLE 1. Strains, plasmids, and phages used in this study.

Strain, plasmid, or phage	Relevant characteristics	Source or reference
E. coli		
1100	Endonuclease I	13
DH5α	*supE44 ΔlacU169* (φ80 *lacZ*ΔM15) *hsdR17 recA1 gyrA96 thi-1 relA1*	18
GB802	*trmA5 argH1 recA1*	35
GB1-5-797	*trmA argH*	This laboratory
K37	Str^r *galK2*	D. Friedman, University of Michigan, Ann Arbor
S. typhimurium GT344	*hisO1242 hisD6404*	J. Roth, University of Utah, Salt Lake City
pBR322	Ap^r Tc^r	6
pBluescript SK	Ap^r *lacZ*$^+$	Stratagene
pGEM4	Ap^r T7 RNA polymerase promoter	Promega Biotec
pGP100	Ap^r *trmA*$^+$	28
pGP200	Ap^r Cm^r *trmA*	28
pGP1843	Ap^r *trmA*$^+$ *argH*$^+$	This laboratory
pGP1509	Ap^r *trmA*$^+$	This laboratory
pUST105	Neo^r *argH*$^+$ *trmA*$^+$	This laboratory
pUST106	Ap^r *trmA*$^+$	This laboratory
pKH3-3	Ap^r *btuB*$^+$	21
pCG11	Ap^r *trmA*$^+$	This laboratory
pCG13	Ap^r Cm^r *trmA*	This laboratory
pCG14	Ap^r T7 RNA polymerase promoter for synthesis of antisense RNA probe	This laboratory
pLG339	Neo^r Tc^r	42
λd*arg*13	*argH*$^+$ *trmA*$^+$	10

downstream of the T7 promoter. This plasmid was constructed by digesting pGP100 (*trmA*$^+$) with *Bss*HII and blunting the sticky end with Klenow fragment. The plasmid was then digested with *Eco*RI, and the 215-bp fragment containing the *trmA* promoter was isolated on NuSieve agarose (FMC BioProducts, Rockland, Maine). This fragment was ligated into *Eco*RI-*Hin*dIII-linearized pGEM4 (Promega Biotec, Madison, Wis.). The *Hin*dIII sticky end of pGEM4 had been blunted with Klenow fragment. Labeled in vitro transcripts from plasmid pCG14 were synthesized by using T7 RNA polymerase according to the supplier's recommendations (Pharmacia-LKB Biotechnology), generating antisense RNA spanning the promoter, which was used as a probe for T2 mapping of the 5′ end.

The probe used in S1 mapping of the 3′ end was made by using the *Sal*I (position 1279)-*Pvu*II (position 1684) fragment from the 3′ end of *trmA* that had been eluted in double-stranded form from a 5% polyacrylamide gel by displacement electrophoresis (38). The probe was labeled by filling in overhanging 5′ ends with Klenow fragment and labeled deoxynucleoside triphosphates. For overproduction of *trmA* transcripts, plasmid pCG11 was constructed by inserting the *Hin*dIII-*Pvu*II fragment containing the entire *trmA* gene into plasmid pBluescript SK. Total cellular RNA from strain 1100 with or without pCG11 was recovered by extraction with hot phenol (48). Hybridization was carried out for 3 h at melting temperature (T_m) for the different probes in hybridization buffer (39). After hybridization, samples for 5′ mapping were diluted 12-fold in nuclease T2 buffer by the method of Costa et al. (8) and incubated at 30°C for 2 h with 18 U of nuclease T2 per sample (Bethesda Research Laboratories). The nuclease T2 treatment was repeated once with an additional 9 U of nuclease T2.

Samples for 3′ mapping were chilled on ice after hybridization to the *Sal*I-*Pvu*II probe as described above and diluted fivefold in S1 nuclease buffer (39) containing 3,000 U of nuclease S1 per sample (Boehringer GmbH, Mannheim, Germany). Samples were incubated at 37°C for 2 h. The S1 treatment was repeated once with an additional 1,000 U of S1 for 1.5 h following extraction with phenol and precipitation with ethanol. After ethanol precipitation, samples were run on 6% polyacrylamide sequencing gels with a sequencing ladder as the size standard (see Fig. 3).

Transcriptional regulation of the *trmA-cat* fusion. The transcriptional growth rate dependency of *trmA* gene expression was measured by a transcriptional chloramphenicol acetyltransferase (CAT) gene fusion (*cat* fusion). Plasmid pCG13, containing a transcriptional *trmA-cat* fusion as well as 2.1 kb of DNA upstream of the *trmA* promoter, was used in all CAT expression determinations. Plasmid pCG13 was constructed by inserting the *Eco*RI-*Pvu*II fragment of plasmid pGP200 spanning the *trmA-cat* fusion transcript into *Eco*RI-*Pvu*II-digested pGP1509. A fragment containing 1.7 kb of the *trmA* upstream region from plasmid pKH3-3 (kindly supplied by R. J. Kadner) was inserted in front of the *trmA* gene. Strain K37 was used as a host in all CAT expression determinations.

CAT activity was measured by the method of Kingston and Sheen (25). Each datum point of CAT activity is given as a percentage of acetylated radioactive chloramphenicol of the total amount added and represents the average of duplicate CAT activity measurements with 0.5 and 0.25 μg of crude protein extract for each growth rate. Protein extracts were prepared by four freeze-thaw cycles for each sample. The CAT specific activity was normalized to a copy number of 14 plasmid copies per genome, which is the same as that shown by the culture grown in rich media (k = 1.4). Cultures grown at k = 0.8 had 17 copies per genome, those grown at k = 0.6 had 31 copies per genome, and those grown at k = 0.3 had 36 copies per genome. Protein was determined by the Bio-Rad protein assay according to the manufacturer's recommendations, with bovine serum albumin as a standard. The copy number of plasmid pCG13 in the different media was measured in quadruplet according to Taylor and Brose (45).

Nucleotide sequence accession numbers. The sequence data (Fig. 2) are available from EMBL/GenBank/DDBJ under accession no. M57568 (*trmA* from *E. coli*) and M57569 (*trmA* from *S. typhimurium*).

RESULTS

DNA sequence of the *trmA* gene of *E. coli*. The complete nucleotide sequence and its translation into amino acids are presented (Fig. 2). We identified an open reading frame from positions 384 to 1481 as the *trmA* gene by comparing the amino acid sequence deduced from the DNA sequence with the previously determined sequence of the NH_2-terminal part of the protein (37). The *trmA* open reading frame is preceded by a putative ribosome binding site, AGGA, and shows the general characteristics of *E. coli* translation initiation sites (43). The DNA sequence indicates that the *trmA* gene would encode a 42,112-Da polypeptide containing 366 amino acids. This is in good agreement with the apparent molecular mass of 42 kDa observed from sodium dodecyl sulfate-polyacrylamide gel analysis of the purified enzyme (37) and extracts from minicells harboring *trmA*$^+$ plasmids (28). Furthermore, the experimentally determined amino acid composition (37) is consistent with the amino acid sequence (Fig. 2). The 202 nucleotides (nt) downstream from the *trmA* structural gene did not contain any open reading frames in either direction.

Two amino acid sequence motifs in DNA methyltransferases have been pointed out previously (26). One of them, -$^{Glu}_{Asp}$-X-Phe-X-Gly-X-Gly-, where X stands for any amino acid, was suggested to be the *S*-adenosylmethionine binding site, and indeed, at amino acids 216 to 222, the *trmA* gene product has the sequence -Glu-Leu-Tyr-Cys-Gly-Asn-Gly-. This sequence is in agreement with the suggested *S*-adenosylmethionine binding site (underlined amino acids), except that in the second position of the sequence motif a hydrophobic and aromatic amino acid, phenylalanine, is substituted by the likewise hydrophobic and aromatic amino acid tyrosine.

The DNA sequence presented in this paper overlaps by 322 nt the sequence of the *btuB* gene (21), which encodes an outer membrane receptor protein for vitamin B_{12}. When comparing the sequences, we found discrepancies in two positions (positions 161 and 320), both of which were resequenced by us several times with no ambiguities. The fact that the *btuB* gene is transcribed from its own promoter in the direction opposite to that of *trmA* (1) and the transcriptional mapping presented below suggest that the promoter identified by us is the *trmA* promoter in vivo.

The DNA sequence 9 nt downstream of the translation stop codon for the tRNA(m^5U54)methyltransferase polypeptide reveals a region of dyad symmetry (positions 1489 to 1514), which could form a stable secondary RNA structure (ΔG = −20.4 kcal [ca. −85.6 kJ]), and suggests a functional role as a transcription termination signal. However, it should be noted that this possible termination structure is not

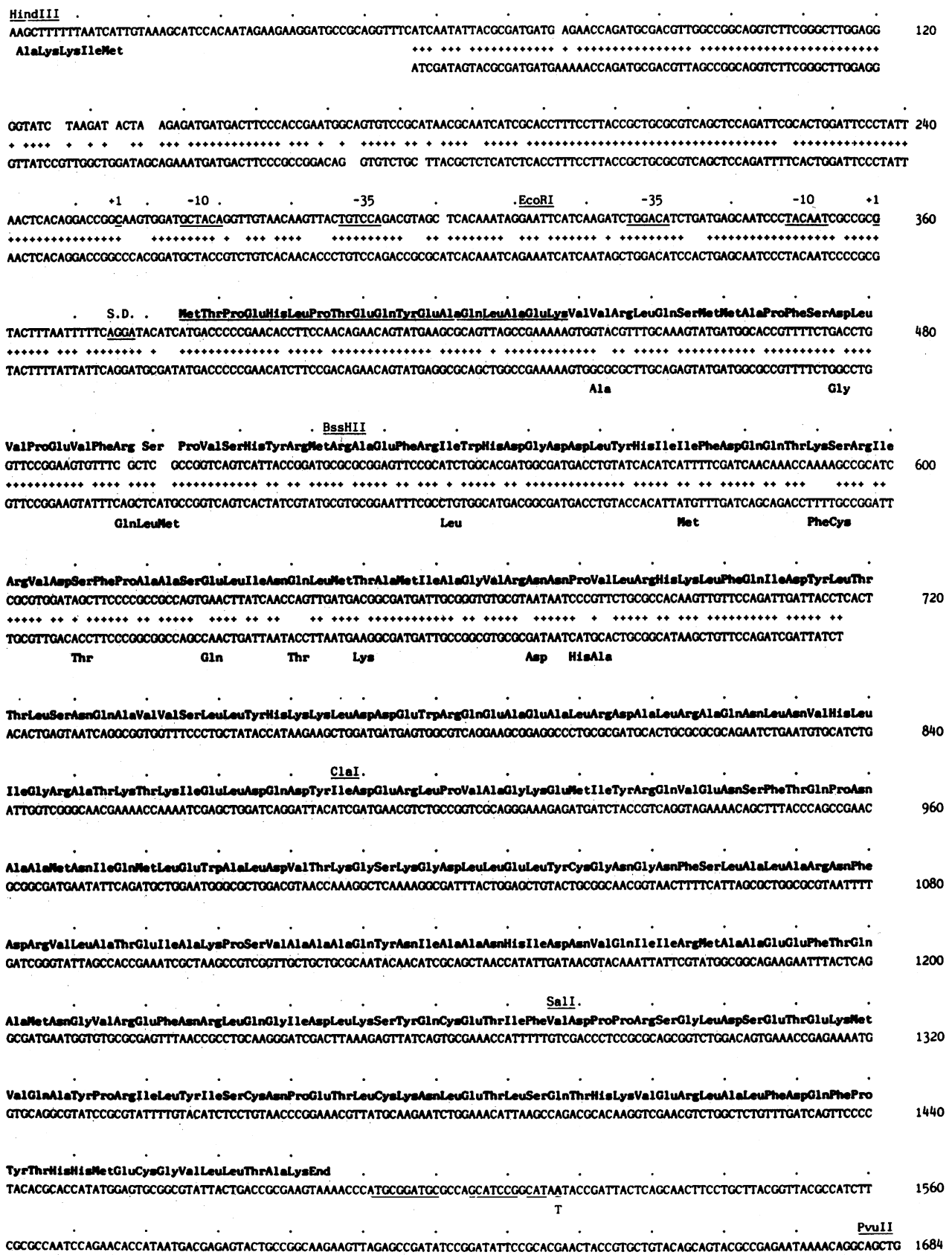
HindIII
AAGCTTTTTTAATCATTGTAAAGCATCCACAATAGAAGAAGGATGCCGCAGGTTTCATCAATATTACGCGATGATG AGAACCAGATGCGACGTTGGCCGGCAGGTCTTCGGGCTTGGAGG 120
AlaLysLysIleMet
ATCGATAGTACGCGATGATGAAAAACCAGATGCGACGTTAGCCGGCAGGTCTTCGGGCTTGGAGG
GGTATC TAAGAT ACTA AGAGATGATGACTTCCCACCGAATGGCAGTGTCCGCATAACGCAATCATCGCACCTTTCCTTACCGCTGCGCGTCAGCTCCAGATTCGCACTGGATTCCCTATT 240
GTTATCCGTTGGCTGGATAGCAGAAATGATGACTTCCCGCCGGACAG GTGTCTGC TTACGCTCTCATCTCACCTTTCCTTACCGCTGCGCGTCAGCTCCAGATTTTCACTGGATTCCCTATT
+1 -10 -35 EcoRI -35 -10 +1
AACTCACAGGACCGGCAAGTGGATGCTACAGGTTGTAACAAGTTACTGTCCAGACGTAGC TCACAAATAGGAATTCATCAAGATCTGGACATCTGATGAGCAATCCCTACAATCGCCGCG 360
AACTCACAGGACCGGCCCACGGATGCTACCGTCTGTCACAACACCCTGTCCAGACCGCGCATCACAAATCAGAAATCATCAATAGCTGGACATCCACTGAGCAATCCCTACAATCCCCGCG
S.D. MetThrProGluHisLeuProThrGluGlnTyrGluAlaGlnLeuAlaGluLysValValArgLeuGlnSerMetMetAlaProPheSerAspLeu
TACTTTAATTTTTCAGGATACATCATGACCCCCGAACACCTTCCAACAGAACAGTATGAAGCGCAGTTAGCCGAAAAAGTGGTACGTTTGCAAAGTATGATGGCACCGTTTTCTGACCTG 480
TACTTTTATTATTCAGGATGCGATATGACCCCCGAACATCTTCCGACAGAACAGTATGAGGCGCAGCTGGCCGAAAAAGTGGCGCGCTTGCAGAGTATGATGGCGCCGTTTTCTGGCCTG
Ala Gly
BssHII
ValProGluValPheArg Ser ProValSerHisTyrArgMetArgAlaGluPheArgIleTrpHisAspGlyAspAspLeuTyrHisIleIlePheAspGlnGlnThrLysSerArgIle
GTTCCGGAAGTGTTTC GCTC GCCGGTCAGTCATTACCGGATGCGCGCGGAGTTCCGCATCTGGCACGATGGCGATGACCTGTATCACATCATTTTCGATCAACAAACCAAAAGCCGCATC 600
GTTCCGGAAGTATTTCAGCTCATGCCGGTCAGTCACTATCGTATGCGTGCGGAATTTCGCCTGTGGCATGACGGCGATGACCTGTACCACATTATGTTTGATCAGCAGACCTTTTGCCGGATT
GlnLeuMet Leu Met PheCys
ArgValAspSerPheProAlaAlaSerGluLeuIleAsnGlnLeuMetThrAlaMetIleAlaGlyValArgAsnAsnProValLeuArgHisLysLeuPheGlnIleAspTyrLeuThr
CGCGTGGATAGCTTCCCCGCCGCCAGTGAACTTATCAACCAGTTGATGACGGCGATGATTGCGGGTGTGCGTAATAATCCCGTTCTGCGCCACAAGTTGTTCCAGATTGATTACCTCACT 720
TGCGTTGACACCTTCCCGGCGGCCAGCCAACTGATTAATACCTTAATGAAGGCGATGATTGCCGGCGTGCGCGATAATCATGCACTGCGGCATAAGCTGTTCCAGATCGATTATCT
Thr Gln Thr Lys Asp HisAla
ThrLeuSerAsnGlnAlaValValSerLeuLeuTyrHisLysLysLeuAspAspGluTrpArgGlnGluAlaGluAlaLeuArgAspAlaLeuArgAlaGlnAsnLeuAsnValHisLeu
ACACTGAGTAATCAGGCGGTGGTTTCCCTGCTATACCATAAGAAGCTGGATGATGAGTGGCGTCAGGAAGCGGAGGCCCTGCGCGATGCACTGCGCCGCGCAGAATCTGAATGTGCATCTG 840
ClaI.
IleGlyArgAlaThrLysThrLysIleGluLeuAspGlnAspTyrIleAspGluArgLeuProValAlaGlyLysGluMetIleTyrArgGlnValGluAsnSerPheThrGlnProAsn
ATTGGTCGGGCAACGAAAACCAAAATCGAGCTGGATCAGGATTACATCGATGAACGTCTGCCGGTCGCAGGGAAAGAGATGATCTACCGTCAGGTAGAAAACAGCTTTACCCAGCCGAAC 960
AlaAlaMetAsnIleGlnMetLeuGluTrpAlaLeuAspValThrLysGlySerLysGlyAspLeuLeuGluLeuTyrCysGlyAsnGlyAsnPheSerLeuAlaLeuAlaArgAsnPhe
GCGGCGATGAATATTCAGATGCTGGAATGGGCGCTGGACGTAACCAAAGGCTCAAAAGGCGATTTACTGGAGCTGTACTGCGGCAACGGTAACTTTTCATTAGCGCTGGCGCGTAATTTT 1080
AspArgValLeuAlaThrGluIleAlaLysProSerValAlaAlaAlaGlnTyrAsnIleAlaAlaAsnHisIleAspAsnValGlnIleIleArgMetAlaAlaGluGluPheThrGln
GATCGGGTATTAGCCACCGAAATCGCTAAGCCGTCGGTTGCTGCTGCGCAATACAACATCGCAGCTAACCATATTGATAACGTACAAATTATTCGTATGGCGGCAGAAGAATTTACTCAG 1200
SalI.
AlaMetAsnGlyValArgGluPheAsnArgLeuGlnGlyIleAspLeuLysSerTyrGlnCysGluThrIlePheValAspProProArgSerGlyLeuAspSerGluThrGluLysMet
GCGATGAATGGTGTGCGCGAGTTTAACCGCCTGCAAGGGATCGACTTAAAGAGTTATCAGTGCGAAACCATTTTTGTCGACCCTCCGCGCAGCGGTCTGGACAGTGAAACCGAGAAAATG 1320
ValGlnAlaTyrProArgIleLeuTyrIleSerCysAsnProGluThrLeuCysLysAsnLeuGluThrLeuSerGlnThrHisLysValGluArgLeuAlaLeuPheAspGlnPhePro
GTGCAGGCGTATCCGCGTATTTTGTACATCTCCTGTAACCCGGAAACGTTATGCAAGAATCTGGAAACATTAAGCCAGACGCACAAGGTCGAACGTCTGGCTCTGTTTGATCAGTTCCCC 1440
TyrThrHisHisMetGluCysGlyValLeuLeuThrAlaLysEnd
TACACGCACCATATGGAGTGCGGCGTATTACTGACCGCGAAGTAAAACCCATGCGGATGCGCCAGCATCCGGCATAATACCGATTACTCAGCAACTTCCTGCTTACGGTTACGCCATCTT 1560
T
PvuII
CGCGCCAATCCAGAACACCATAATGACGAGAGTACTGCCGGCAAGAAGTTAGAGCCGATATCCGGATATTCCGCACGAACTACCGTGCTGTACAGCAGTACGCCGAGAATAAAACAGGCAGCTG 1684

FIG. 2. Nucleotide sequences of the complete *trmA* gene from *E. coli* (upper strand) and of the promoter region and N-terminal part of the structural gene from *S. typhimurium* (lower strand). Homologies between the two sequences at the DNA level are indicated (+). Differences in the predicted amino acid level are indicated below the *S. typhimurium* sequence. Underlined amino acids are those determined by N-terminal sequencing of the purified protein (37). The transcriptional and translational start points of the *trmA* and *btuB* genes are also shown (1). The promoters and postulated ribosomal binding sites are underlined and marked (−10, −35 and S.D., respectively). The transcriptional start points are indicated (+1). The major endpoint of the transcript is indicated (T), and the preceding dyad symmetry is underlined. Recognition sequences for restriction enzymes mentioned in the text are also shown. The DNA sequences of both strands were determined, except for some regions for which several independent determinations were made until no uncertainties were encountered.

followed by the poly(U) region characteristic of Rho-independent terminators.

Characterization of transcripts in vivo. Lindström et al. (28) have previously shown that the *Eco*RI-*Pvu*II fragment contains the *trmA* promoter located close to the *Eco*RI site. They showed that the promoter is essential for transcription of the *trmA* gene in vitro. In accordance with the earlier findings, our sequence analysis revealed a consensus promoter structure upstream of the structural gene (Fig. 2), as well as the presence of a possible termination signal in the *Sal*I-*Pvu*II region close to the *Pvu*II site. In order to define the actual start and stop signals of the transcripts produced in vivo, we mapped the 5′ end of the transcript, using both T2 nuclease mapping and primer extension. The results of the T2 nuclease mapping of the 5′ end of the *trmA* transcript are shown (Fig. 3). The 5′ end of the mRNA was localized to position 360 (Fig. 2), which matches the proposed −35 and −10 promoter regions deduced from the DNA sequence. It also matches a typical procaryotic start point of transcription, whose consensus is pyrimidine-purine-pyrimidine, starting with the purine CGT (19). Primer extension confirmed the T2 nuclease results (data not shown). S1 analysis of the 3′ end of the *trmA* transcript with a 3′-labeled *Sal*I-*Pvu*II probe showed two major bands corresponding to nt 1517 and 1522 (Fig. 3) immediately downstream of the dyad symmetry region following the stop codon of the protein.

Our analysis of the *trmA* promoter revealed similarities to the rRNA P1 promoter both upstream and downstream from the −10 region (Fig. 4). Immediately downstream from the −10 region of the *trmA* promoter is a sequence, CGCCGCG, similar to the discriminatory region for stringently controlled promoters (46), although the *trmA* promoter extends the GC base pairing with 3 extra GC bp (GCG) compared with the 2 bp ($^{A}_{T}$CC) of the rRNA P1 promoter. In addition, we found a conserved sequence motif, TCCC, immediately upstream from the −10 region, previously found only in the P1 promoters of rRNA genes and some tRNA promoters (Fig. 4). A sequence similar to the *E. coli* factor for inversion stimulation (FIS) binding site (22) was found at positions 299 to 308 between the −35 regions of the *trmA* and *btuB* promoters (7 of 9 bp). FIS binding sites have also been found in the *rrn* promoters and are centered there at about −71, i.e., somewhat further upstream than the putative FIS binding site in the *trmA* promoter, which is centered at −56 (32, 47).

Cloning and sequence analysis of the *trmA* gene from *S. typhimurium*. To get an indication of the evolutionary conservation of the *trmA* promoter elements which are homologous with rRNA P1 promoters, we cloned the *trmA* gene from *S. typhimurium* and compared the promoter sequences of the two species. A genomic library from *S. typhimurium* was used to clone the *trmA* gene by selecting Arg^+ transformants in an *argH* auxotrophic strain. We thereby located the *trmA* gene to min 89, which is analogous to the map position in *E. coli*. The nucleotide sequence of the *trmA* gene from *S. typhimurium* showed that the discriminatory region in the promoter contains the sequence CCCC with the extended

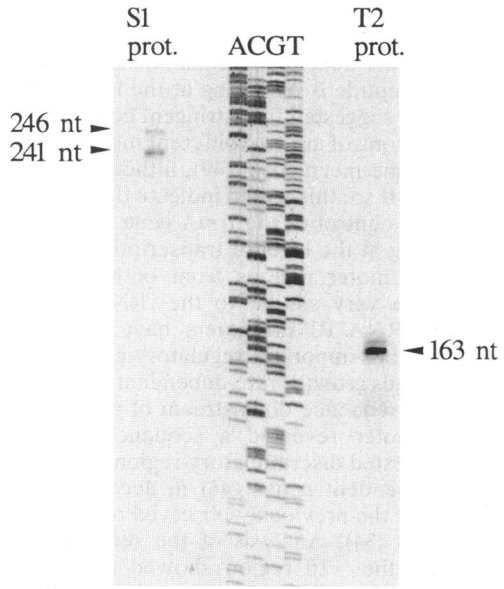

FIG. 3. Mapping of the *trmA* transcript. Results show the nuclease T2-protected fragment (163 nt) with the 5′ end mapped and the nuclease S1-protected fragments (241 and 246 nt) with the 3′ end of *trmA* mRNA mapped. The band corresponding to 241 nt represents the major 3′ end. Also included is a sequencing ladder utilized as a size standard.

			-35	-10
E. coli	trmA	P	TGGACA--12nt--TCCCTACAAT	CGCCGCGTA
S. typhimurium	trmA	P	TGGACA--12nt--TCCCTACAAT	CcCCGCGTA
E. coli	rrnA	P1	TtGtCA--12nt--TCCCTAtAATgCGCCaCcac	
E. coli	rrnB	P1	TtGtCA--12nt--TCCCTAtAATgCGCCaCcac	
E. coli	rrnC	P1	TtGtCA--12nt--TCCCTAtAATgCGCCaCcac	
E. coli	rrnD	P1	TtGtgc--12nt--TCCCTAtAATgCGCCtCcgt	
E. coli	rrnE	P1	TtGcgg--12nt--TCCCTAtAATgCGCCtCcat	
E. coli	rrnG	P1	TtGtCA--12nt--TCCCTAtAATgCGCCaCcac	
E. coli	rrnH	P1	TtGtCt--12nt--TCCCTAtAATgCGCCtCcat	

FIG. 4. Comparison of the *trmA* promoter with the seven rRNA P1 promoters. Capital letters in the *S. typhimurium trmA* and *rrn* promoter sequences indicate identical nucleotides. −35 and −10 indicate the location in the promoter. The start point of transcription is underlined. Sequences for the rRNA P1 promoters are based on those of Jinks-Robertson and Nomura (23).

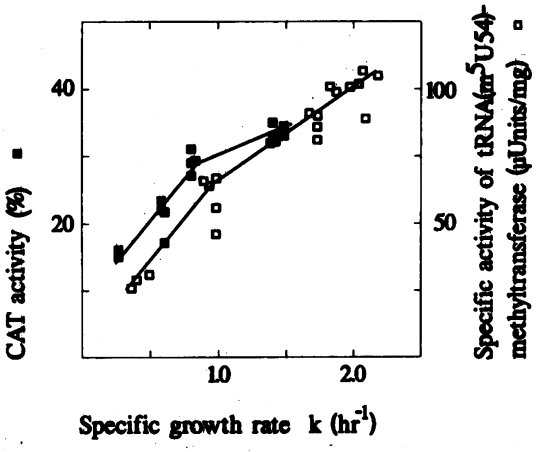

FIG. 5. Effect of growth rate on the regulation of the *trmA* promoter. CAT activity (■) and tRNA(m^{5}U54)methyltransferase activity (□) on the *y* axes are plotted as a function of specific growth rate on the *x* axis. CAT activity is given as percent aminoacetylated [^{14}C]chloramphenicol of total amount added to the sample. Each datum point of CAT activity represents the average of two CAT activity measurements in a single experiment at each growth rate. The tRNA(m^{5}U54)methyltransferase activity is based on the findings of Ny and Björk (35).

GC base pairing GCG (Fig. 4). The sequence upstream from the −10 region is identical to that in *E. coli* and in the rRNA P1 promoter, i.e., TCCC. The nucleotide sequence of *trmA* of *S. typhimurium* was 81% homologous to the nucleotide sequence of *trmA* of *E. coli* (523 of 660 nt). On the amino acid level, the homology was 86% (95 of 111 amino acids) (Fig. 2).

Transcriptional regulation of the *trmA* operon. Previous studies of the regulation of *trmA* gene expression, demonstrating the growth rate dependency and stringent control, have all measured the amount or activity of the polypeptide (34, 35). The similarity of the *trmA* promoter to the *rrn* P1 promoters, together with the similar regulatory behavior of *trmA* and *rrn* gene expression, prompted us to measure the transcription of the *trmA* gene. *cat* operon fusions have previously been shown to be a true measurement of transcriptional activity (27). Therefore, an operon fusion between *cat* and *trmA* was constructed. *cat* gene activity, protein concentration, and copy number were determined at different growth rates (Fig. 5). The specific CAT activity given as a function of the specific growth rate correlates to that of the tRNA(m^{5}U54)methyltransferase specific activity (k ranging from 0.3 to 1.5 h^{-1}). Thus, the growth rate-dependent regulation of the *trmA* gene is operating at the level of transcription.

The *btuB* and *trmA* promoters are divergent promoters arranged back to back and separated by 102 nt of intervening DNA between the respective starts of transcription. This is a relatively short distance compared with those of other divergent promoters and could provide the opportunity for some regulatory factor(s) to interact with both promoters (3). The structural gene *btuB* codes for the outer membrane binding protein in the vitamin B_{12} (cobalamin) transport system (21). Gene expression of the BtuB polypeptide has been shown to be repressed when cells are grown in the presence of cobalamin (24). Interestingly, it has also been shown that vitamin B_{12} is involved in the modification of tRNA (14). We therefore measured the *trmA* gene activity in cells grown in different cobalamin concentrations as described by Kadner (24). However, we did not detect any significant difference in the level of tRNA(m^{5}U54)methyltransferase specific activity under those conditions (data not shown). It therefore appears that the two operons are regulated independently of each other.

DISCUSSION

The results presented in this paper demonstrate that the *trmA* gene is transcribed in vivo from the promoter at position 360 (Fig. 1). The 3′ end of the *trmA* transcript was mapped to a position immediately downstream of a dyad symmetry region of 9 bp with one internal mismatch. This possible secondary RNA structure is not followed by a poly(U) stretch common to Rho-independent termination signals. In previous in vitro transcription experiments using a purified RNA polymerase, extensive transcription through this region was observed (28). Thus, the above observations suggest either a possible factor-dependent termination of *trmA*-specific transcription or a processing site. However, we have previously shown that several Tn*5* insertions in the *trmA* gene influence only the synthesis of the *trmA* gene product and not the synthesis of the two downstream polypeptides as demonstrated with minicells (28). Furthermore, even after extensive exposure of the autoradiogram, no transcript larger than the one with the indicated 3′ end was observed (unpublished results). Therefore, if the 3′ end is generated through a processing event, the larger precursor must be very unstable, and the gene products encoded downstream on the *trmA* transcript are not detected in a minicell experiment. Although the possibility of a processing event is not entirely excluded, our results are consistent with the *trmA* gene being monocistronic, unlike all other genes for tRNA-modifying enzymes hitherto sequenced (5).

In order to monitor regulation of the *trmA* operon, a transcriptional fusion with the *cat* gene was constructed. The growth rate-dependent gene activity was strikingly similar when the *cat* gene fusion as a measurement of *trmA* transcription was compared with the tRNA(m^{5}U54)methyltransferase activity itself (Fig. 5). We therefore conclude that the growth rate-dependent regulation of the synthesis of the TrmA polypeptide is operating at the level of transcription. It has been suggested that stringent control and growth rate-dependent control are two different modes of regulation based on the same mechanism (49), although this has been questioned (16). If so, this would indicate that the previously shown stringent control of the *trmA* gene expression could also be operating at the level of transcription.

The *trmA* promoter regions from both *E. coli* and *S. typhimurium* are very similar to the rRNA P1 promoters (Fig. 4). The rRNA P1 promoters have previously been suggested to harbor important regulatory elements for stringent control and growth rate-dependent regulation (23). Analysis of the sequence downstream of the −10 region of the *trmA* promoter revealed a sequence similar to the previously suggested discriminatory region for stringent and growth rate-dependent genes (46) in accordance with the present data and the previously observed regulation of *trmA* gene expression (34). Analysis of the sequence extending upstream from the −10 region showed an additional sequence homology to rRNA P1 promoters. To our knowledge, the conserved sequence TCCC in both species has not been previously identified in any other σ^{70} promoter (i.e., other than rRNA promoters and some tRNA promoters), and its presence raises the possibility of an additional regulatory determinant that may be important for these

promoters. Interestingly, a similar sequence, CCCC, is found in the corresponding position in promoters transcribed by the heat shock sigma factor σ^{32} (9). These nucleotides are in close contact with the RNA polymerase during open complex formation at the start of transcription (41), which is why this conserved sequence might be important in some aspect of the regulation which is similar in the *trmA* and *rrn* promoters. Dickson et al. (12) and Gaal et al. (15) have changed two of the four bases in the TCCC sequence of *rrnB* P1, resulting in TTCC (C-17T) and CCCC (T-18C). The two promoter mutants were assayed for activity and growth rate-dependent regulation, and they showed decreases in absolute activity of 55 and 16%, respectively. The growth rate dependency was unchanged compared with that of the wild type in the case of T-18C. However, mutant C-17T showed a reduced growth rate-dependent response. A double mutant (C-1 to T and C-15 to G) totally eliminates growth rate control. Thus, this conserved TCCC sequence is indeed involved in growth rate-dependent regulation, although other features of the promoter are also important in this regard.

Among protein-encoding genes, the *trmA* gene seems unique in that its expression is regulated in a fashion similar to that of *rrn* and some tRNA genes. There are several proteins, e.g., r-proteins, aminoacyl-tRNA ligases, EF-Tu, EF-G, and glucose-6-phosphate dehydrogenase (*gnd*), which are synthesized in a manner coordinate to that of rRNA (7, 17, 33). However, in each of these cases, it has been shown that the regulatory mechanism achieving this coordinate regulation is different from that governing the regulation of rRNA. In the case of *trmA*, the mechanism governing the growth rate-dependent regulation is likely to be the same as that of rRNA. Thus, the *trmA* mRNA constitutes a novel example of an mRNA regulated through a mechanism similar or identical to that of stable RNA.

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