JOURNAL OF BACTERIOLOGY, Jan. 1998, p. 359–365 Vol. 180, No. 2
0021-9193/98/$04.00+0

Identification of the *rrmA* Gene Encoding the 23S rRNA m^1G745 Methyltransferase in *Escherichia coli* and Characterization of an m^1G745-Deficient Mutant

CLAES GUSTAFSSON[1]† AND BRITT C. PERSSON[2]*

Sinsheimer Laboratories, University of California, Santa Cruz, California 95064,[1] and Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden[2]

Received 23 September 1997/Accepted 6 November 1997

An *Escherichia coli* mutant lacking the modified nucleotide m^1G in rRNA has previously been isolated (G. R. Björk and L. A. Isaksson, J. Mol. Biol. 51:83–100, 1970). In this study, we localize the position of the m^1G to nucleotide 745 in 23S rRNA and characterize a mutant deficient in this modification. This mutant shows a 40% decreased growth rate in rich media, a drastic reduction in loosely coupled ribosomes, a 20% decreased polypeptide chain elongation rate, and increased resistance to the ribosome binding antibiotic viomycin. The *rrmA* gene encoding 23S rRNA m^1G745 methyltransferase was mapped to bp 1904000 on the *E. coli* chromosome and identified to be identical to the previously sequenced gene *yebH*.

Mature 23S rRNA from *Escherichia coli* contains some 23 modified nucleotides, of which 14 are methylations of the base or the sugar moiety of the nucleotide (2). In general, the locations of the modified nucleotides in the 23S rRNA secondary structure correlate well with those of universally conserved nucleotides (13), and the nucleotides are clustered within the proposed three-dimensional structure of 23S rRNA at the peptidyltransferase center, the active center of the molecule (6). So far, the only 23S rRNA-modifying enzyme that has been cloned in *E. coli* is the 23S Ψ746 synthase (36).

In the early work by Björk and Isaksson (3), a number of RNA modification-deficient mutants of *E. coli* were isolated by screening for RNA possessing methyl group acceptor ability in vitro. One of the identified mutant strains, IB10, lacked the modified nucleoside m^1G in the rRNA, but the location of the modification was not further mapped. The mutation was denoted *rrmA* (ribosomal RNA methyltransferase A). The enzyme, 23S rRNA m^1G745 methyltransferase, has been partially purified, and its substrate requirements have been analyzed (17, 18). Strains lacking m^2G and m^5C in the rRNA were also identified (3, 4). These mutant strains can be used to characterize the function of rRNA modifications and to identify the gene encoding the corresponding RNA modifying enzyme.

In this study, we localize the modified nucleotide on the rRNA, identify the gene (*rrmA*) encoding the rRNA-modifying enzyme, and analyze the growth characteristics and susceptibility to antibiotics of an *rrmA* mutant.

MATERIALS AND METHODS

Strains, chemicals, and standard protocols. All the strains used were derivatives of *E. coli* K-12 and are listed in Table 1. Strain IB10 is the original isolate from the screening of RNA methylation-deficient mutants. It is a ethyl methanesulfonate-mutagenized derivative of strain CP79. The mutant allele of the gene encoding 23S rRNA m^1G745 methyltransferase (*rrmA10*) was crossed into a nonmutagenized CP79 background by conjugation, resulting in strain IB103. These strains were kindly provided by Glenn Björk, University of Umeå, Umeå, Sweden. Transductions and conjugations were done by established methods (24). Standard molecular biology procedures were those described by Sambrook et al. (29). Viomycin was generously provided by Nathan Belcher, Pfizer. Reverse transcriptase was purchased from Seikagaku.

HPLC analysis. The 30S and 50S ribosomal subunits were separated on a sucrose gradient, and rRNA was extracted by phenol as described previously (32). The RNA samples were digested to nucleosides with nuclease P1 (Boehringer) and bacterial alkaline phosphatase (Sigma) (11). After centrifugation, 100 μg of rRNA nucleosides was applied to a Supelcosil LC-18S column on a Waters high-pressure liquid chromatography (HPLC) system. The gradient used was that of Gehrke and Kuo (10), and the flow rate was 1 ml/min. The absorbance of the samples was monitored at 254 nm. The identity of each HPLC-isolated nucleoside was determined by measuring its retention time and by spectral analysis. The relative amount of each nucleoside was determined by using the relative molar response factor of each nucleoside at 254 nm (10) multiplied by the integrated area under each peak.

Antibiotic resistance. The antibiotics viomycin, erythromycin, celesticetine, carbomycin, chloramphenicol, vernamycin B, thiostrepton, and streptogramin B were each dissolved to saturation. Filter paper (Whatman GFC) was soaked in the antibiotic solution and placed on rich-medium plates containing the appropriate strain. The zone of growth inhibition from the filter was determined. For further characterization of the level of viomycin resistance, strains were spread on Luria-Bertani (LB) media plates containing 0 to 300 μM viomycin and incubated overnight at 37°C.

Mutant screening. *E. coli* CP79 was spread at a density of 5,000 colonies per rich-medium plate containing 150 μM viomycin and incubated overnight at 37°C. Spontaneous Vio^r mutants were streaked on LB medium and then restreaked on LB medium containing 150 μM viomycin to avoid chromosomal duplications.

Chemical protection and primer extension. The 50S ribosomal subunits (5 pmol, 100 nM) were incubated with 0.1 μM to 10 mM viomycin in 50 μl of 80 mM potassium-cacodylate (pH 7.2)–75 mM NH_4Cl–1 mM dithiothreitol–0.5 mM EDTA–15 mM $MgCl_2$–75 mM KCl for 30 min at 37°C followed by 10 min on ice. The complexes were modified with kethoxal (5 μl of a 1:5 [vol/vol] dilution in H_2O) at 37°C for 8 min. The reaction was stopped, and rRNA was extracted. Primer extension reactions and gel electrophoresis were carried out as described previously (25). Primer extension was performed with a 17-mer oligonucleotide which primes reverse transcriptase at nucleotide 872 of 23S rRNA. The gels were scanned and signals were quantified with a PhosphorImager (Molecular Dynamics).

RESULTS

Localization of m^1G to position 745 in 23S rRNA. HPLC analysis of RNA nucleosides was performed to identify the location of the *rrmA*-derived m^1G. Strain CP79- and IB103-derived tRNA and 16S and 23S rRNA were isolated, extracted, and hydrolyzed separately. HPLC analysis of nucleosides from the 23S (and 5S) rRNA identified 0.9 mol of m^1G per mol of m^2A in strain CP79 ($rrmA^+$), whereas m^1G was completely missing in the HPLC profile of the hydrolyzed 23S rRNA derived from the *rrmA* strain IB103 (a derivate of the original

* Corresponding author. Present address: Howard Hughes Medical Institute at University of Utah, 15 N, 2030 E, Rm. 6160, Salt Lake City, UT 84112-5330. Phone: (801) 581-4429. Fax: (801) 585-3910. E-mail: bpersson@genetics.utah.edu.

† Present address: Kosan Biosciences, Inc., Burlingame, CA 94010.

TABLE 1. Strains used in this study

Strain	Genotype or phenotype	Reference
CAG5051	Hfr H, *relA1 thi-1 spoT supQ80 nadA-57*::Tn*10*	31
CAG5054	Hfr KL96, *relA1 thi-1 trpB83*::Tn*10*	31
CAG5055	Hfr KL16, *relA1 thi-1 zed-3069*::Tn*10*	31
CAG8160	Hfr KL14, *relA1 leu thi-39*::Tn*10*	31
CAG8209	Hfr KL228, *thi-1 leu gal-6 supE44 lacY1* or *lacZ4 zgh-3075*::Tn*10*	31
CP79	*thr leu his argA relA*	7
GRB1394	Hfr P4x, *zea-225*::Tn*10 rrmA10*	This study
GRB1398	Hfr P4x, *zea-225*::Tn*10*	This study
IB10	*thr leu his argA relA rrmA10*	3
MW100	Hfr P4x	35
IB103	*thr leu trp his* Str^r *ampA1 rrmA10*	Glenn Björk

TABLE 2. Relative molar amounts of modified nucleosides as determined by HPLC[a]

Nucleoside	Literature value[b]	Amt[c] of nucleoside in:				
		CP79	IB103	GRB1398	GRB1394	GRB1394 (pBP51)
Gm	0.9	1.0	0.7	1.1	1.1	1.1
m^1G	0.8	0.9	<0.01	1.0	<0.01	1.1
m^2A	0.9	1.0	1.0	1.0	1.0	1.0
m^6A	2.1	2.1	2.2	2.2	1.8	2.1

[a] See also Fig. 1.
[b] Literature values for moles of nucleoside per mole of 23S are from reference 10.
[c] Amounts of nucleoside are given as moles of nucleoside per mole of the nucleoside m^2A.

IB10 strain [Fig. 1; Table 2]). The identity of each peak was determined by measuring its retention time and absorbance spectrum. HPLC analysis of nucleosides from tRNA and 16S rRNA showed no difference between the two strains.

A single m^1G has previously been mapped to position 745 of 23S rRNA (15). Primer extension was performed on 23S rRNA isolated from strains IB10 (*rrmA*), IB103 (*rrmA*), and CP79 (*rrmA*$^+$). In this method, synthesis of a complementary DNA strand is initiated by annealing an oligonucleotide to positions 872 to 889 of 23S rRNA. The reverse transcriptase stops synthesizing the DNA strand when it reaches the nucleotide preceding m^1G, since the N-1 of guanosine, required for proper Watson-Crick base pairing to a cytosine, is blocked by a methyl group. As can be seen in Fig. 2, a double band appears at positions corresponding to U746 and G745 in the CP79 lane but is absent in the IB10 and IB103 lanes, showing that the modified nucleotide m^1G, normally found at position 745 of 23S rRNA, is lacking in the *rrmA* strains. The two bands seen at U746 and G745 are likely to reflect transcriptional stuttering as the reverse transcriptase encounters the modified nucleotide. The absence of reverse transcriptase stops at U746, and G745 is the only difference between CP79 and IB103 detected by primer extension over the entire 23S rRNA (data not shown).

The *rrmA* mutants have a decreased growth rate. The growth rate of the isogenic strains GRB1394 (*rrmA*) and GRB1398 (*rrmA*$^+$) was determined in rich LB medium. Strain GRB1394 was found to have a doubling time of 56 min, in

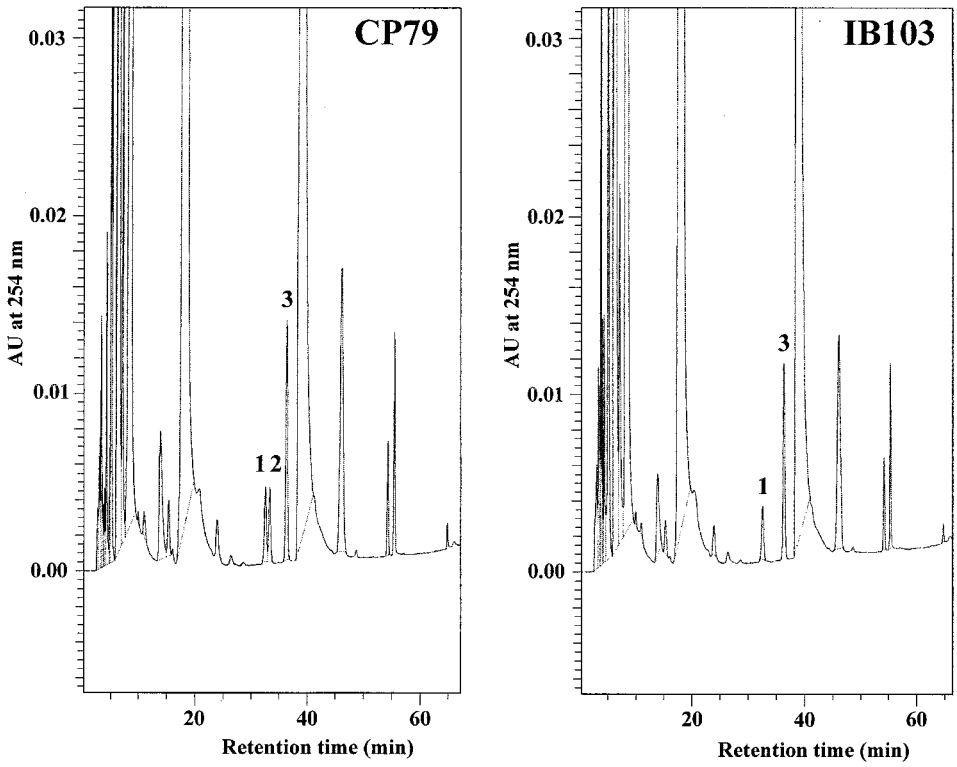

FIG. 1. The modified nucleotide m^1G is lacking in 23S rRNA from strain IB103. HPLC chromatograms of nucleosides from the 50S subunits of strain CP79 (wild type) and strain IB103 (*rrmA*10) are shown. The HPLC chromatogram of RNA from strain IB10 is identical to that of IB103 (data not shown). Peak 1 corresponds to the modified nucleoside Gm, peak 2 corresponds to m^1G, and peak 3 corresponds to m^2G. AU, absorption units.

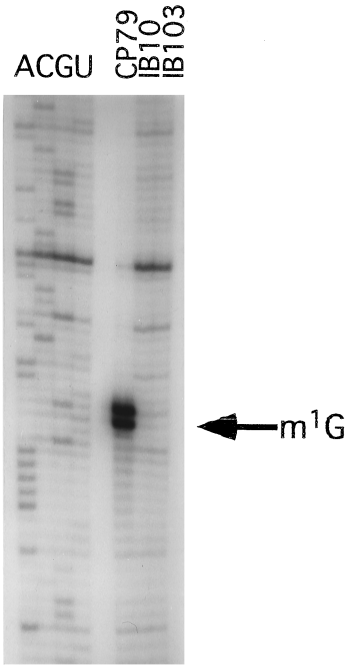

FIG. 2. The *rrmA*-dependent m[1]G modification is located at position 745 of 23S rRNA. The autoradiogram shows reverse transcription of 23S rRNA from strains CP79 (wild type), IB10, and IB103 (both lacking m[1]G745). Also included is RNA sequencing with the same primer as that used for reverse transcription.

contrast to 40 min for the corresponding isogenic strain GRB1398, a difference of 40%.

Polysome profiles and peptide chain growth rate. Strains CP79 and IB103 were grown in rich medium, cells were lysed, and ribosomal subunits and polysomes (i.e., ribosomal complexes sedimenting faster than 70S) were separated by sucrose gradient sedimentation as described previously (28). The separation was done at two different Mg^{2+} concentrations. Regular ribosomal 70S complexes were isolated at 10 mM Mg^{2+}, whereas tightly coupled 70S complexes were identified by exposing the ribosomes to the more stringent 5 mM Mg^{2+}. Under nonstringent conditions (10 mM Mg^{2+}), the *rrmA*$^+$ CP79 cells had 70% of the ribosomal subunits located in the polysomes. The distribution of ribosomal subunits in the *rrmA* mutant IB103 was shown to be distinctly different. Only 20% of the ribosomal subunits in IB103 were found in the polysomes under nonstringent conditions, and there was an increase in the proportion of free 30S and 50S subunits (Fig. 3; Table 2). The relative amounts of tightly coupled ribosomes were identical in the two strains. We believe that the absence of m[1]G745 dissociates the loosely coupled 70S, so that only the tightly coupled 70S ribosomes can be detected in the polysome profile under nonstringent conditions.

The peptide chain elongation rate (cgr_p), i.e., the velocity of the translational cycle as determined by the number of amino acids incorporated into protein per second per ribosome, was determined from strains CP79 and IB103. The cgr_p was determined by measuring the induction time of β-galactosidase as previously described (20). The cgr_p for CP79 was measured to be 14 amino acids per second, which is in the range of the established translational velocity for wild-type *E. coli* of 13 to 16 amino acids per second (19). The cgr_p of the *rrmA* mutant IB103 was determined to 11 amino acids per second, a reduction of approximately 20% (Table 3). The β-galactosidase induction experiment was performed three times for each strain; in each case there was less than a 1-s deviation from the given value of cgr_p.

Gene mapping and complementation. The location of the *rrmA* gene on the *E. coli* chromosome was mapped by using the Hfr strains Hfr H, Hfr KL14, Hfr KL16, Hfr KL96, Hfr KL208, Hfr KL227, and Hfr KL228 (31). The Hfr strains were allowed to conjugate with the *rrmA* strain IB103. A total of 24 conjugants from each conjugation were tested for the presence of m[1]G745 as analyzed by primer extension analysis. Only Hfr KL96, initiating at min 46.6 and with the selected marker at min 28.3, and Hfr KL16, initiating at min 64.5 with the selected marker at min 43.9, could cause the *rrmA* strain to revert to *rrmA*$^+$. In the conjugation with Hfr KL96 as the donor, 11 of 24 conjugants acquired the wild-type *rrmA* gene. In the conjugation with Hfr KL16 as the donor, 14 of 24 conjugants acquired the wild-type *rrmA* gene. None of the other Hfr conjugations could revert the *rrmA* phenotype. The conjugations with Hfr KL96 and Hfr KL16 indicate that the *rrmA* gene is located in the region from 40 to 45 min of the *E. coli* chromosome.

To further narrow the region where *rrmA* is located, strains with Tn*10* insertions between min 37 and 44 were used in P1 transductions with IB103. The following Tn*10* transposons were assayed for cotransduction with *rrmA*: *zdj*-3124::Tn*10kan*, *zea*-225::Tn*10*, *zea*-3068::Tn*10*, *eda*-3126::Tn*10kan*, and *uvrC*279::Tn*10* (31). The transposon insertions *zea*-225::Tn*10* (at min 40.3) and *zea*-3068::Tn*10* (at min 40.9) were both found to cross out the *rrmA* mutation, confirming the map location indicated by Hfr conjugations. Since both transposons were approximately 70% linked to the *rrmA* gene, we conclude that the *rrmA* gene is localized close to these two transposons,

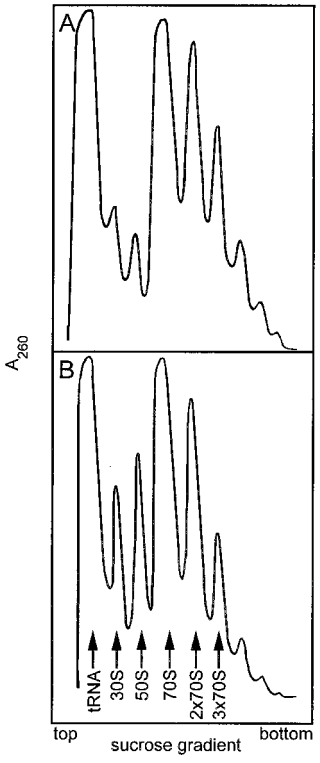

FIG. 3. Sucrose gradient sedimentation profiles of extracts from strains CP79 (A) and IB103 (B) made under nonstringent conditions (10 mM Mg^{2+}). The positions of tRNA, 30S and 50S ribosomal subunits, 70S monosomes, and polysomes are indicated.

TABLE 3. Phenotypic manifestations of *rrmA*

Characteristic	Value in:				
	CP79 (*rrmA*$^+$)	IB103 (*rrmA*)	GRB1398 (*rrmA*$^+$)	GRB1394 (*rrmA*)	GRB1394(pBP51) (*rrmA*/*rrmA*$^+$)
m^1G detected by primer extension	Yes	No	Yes	No	ND[a]
Molar ratio m^1G/m^2A (HPLC)	0.9	<0.01	1.0	<0.01	1.0
Viomycin MIC (μM)	50	200	30	200	ND
Doubling time in rich medium (min)	28	45	40	56	40
Free ribosomal subunits at 6 mM Mg^{2+} (% of total ribosomal subunits)	35	37	ND	ND	ND
Free ribosomal subunits at 10 mM Mg^{2+} (% of total ribosomal subunits)	16	27	ND	ND	ND
cgr_p (aa/s)	14	11	ND	ND	ND
Protection of 50% of G914 from kethoxal modification by viomycin (μM viomycin)	100	0.3	ND	ND	ND

[a] ND, not determined.

around min 40.5 on the genetical map (1). This is approximately equivalent to bp 1900000 on the physical map (5).

The cotransducability of *zea*-225::Tn*10* and *rrmA10* was used to transfer the *rrmA* allele into the otherwise wild-type background of strain MW100. This resulted in strains GRB1394 (*zea*-225::Tn*10 rrmA10*) and the isogenic GRB1398 (*zea*-225::Tn*10 rrmA*$^+$). These two strains were subsequently used in the phenotypic characterization of 23S m^1G745 deficiency.

Identification of the ORF corresponding to *rrmA*. The entire *E. coli* genome is now sequenced, and in the region from bp 1896000 to 1918000, 20 open reading frames (ORFs) have been found. Of these 20 ORFs, 6 have been genotypically characterized (5). Of the remaining 14 ORFs, only 1, *yebH* (f269), has the characteristics of an RNA methyltransferase. The deduced amino acid sequence of *yebH* contains a motif (V-L-D-I-G-C-G-E-G) strikingly similar to the consensus binding site for *S*-adenosylmethionine, the methyl donor for nucleotide methylation (20). The *yebH* ORF also shows homology to *myrA* from *Micromonospora griseorubida* (29% identity at the amino acid level). The *myrA* gene encodes resistance to the antibiotic mycinamicin through an unknown mechanism. The gene *myrB* in the same organism also encodes resistance to this antibiotic and encodes an rRNA 2′-*O*-methyltransferase (16). The *yebH* gene is located downstream of the *cspC* gene at bp 1904000, which corresponds to min 40.9 (21). Plasmid pSJ6 (21) carrying the *yebH* gene together with *cspC* and four uncharacterized ORFs (f47, f95, f47, and f263, where the number indicates the number of amino acids in each ORF) was kindly donated by M. Inouye. None of the uncharacterized ORFs contained an *S*-adenosylmethionine binding motif even when searched at very low stringency; therefore, *yebH* is the only gene on plasmid pSJ6 that can encode a methyltransferase. A plasmid carrying the *yebH* ORF together with its promoter was constructed (pBP51; Fig. 4). Plasmid pBP51 was introduced into strain GRB1394 (*rrmA*) and was shown to complement the slow growth of the *rrmA* mutant and also to restore the m^1G modification of the 23S rRNA (Table 2). Therefore, we conclude that the *yebH* gene corresponds to the *rrmA* locus and encodes the 23S rRNA m^1G745 methyltransferase.

The *rrmA* mutant is resistant to viomycin. Many antibiotics function as specific ribosome binding factors that inactivate the ribosome, and thereby inactivate translation, by interacting with accessible structures. Changing the ribosome surface by removing a hydrophobic methyl group from an exposed nucleotide may affect the binding of different factors, such as antibiotics, to the ribosome. Examples of modified ribosomal nucleotides that alter the level of resistance to various antibiotics include 16S $m^6_2A1518,1519$, which changes the resistance to kasugamycin (14), 23S Am1067, which changes resistance to thiostrepton (34), and 23S m^6_2A2058, which changes resistance to erythromycin (12). Based on this, we asked whether the lack of m^1G745 resulted in altered resistance to any of a number of different antibiotics that have been shown to bind 23S rRNA. Strains CP79, IB10, and IB103 were grown on rich-medium agar plates. A filter soaked in the antibiotic to be tested was placed on each plate, and the plates were incubated at 37°C. The following day, the zone of growth inhibition was determined. Strain CP79 showed a 5-mm clearance zone surrounding the filters soaked in viomycin, whereas strains IB103 and IB10 were not sensitive to the drug (Fig. 5). The remaining tested antibiotics did not exhibit any significant difference in the level of resistance. Further characterization showed that strain CP79 grows on rich-medium plates at 37°C with a viomycin MIC of 50 μM. The viomycin MIC for strains IB10 and IB103 was shown to be 200 μM.

Viomycin protection of rRNA in wild-type and mutant strains. It has previously been shown that viomycin protects G914 of 23S rRNA from chemical modification by kethoxal (26). The relative level of protection by viomycin from chemical modification of CP79-derived 50S and IB103-derived 50S ribosomal subunits was determined. The concentration of viomycin was determined in the presence of the 50S subunits. The rRNA was chemically modified by addition of kethoxal and isolated by phenol extraction. Kethoxal attacks and blocks exposed N-1 and N-2 of guanosine to prevent base pairing. The blocked G can then be identified by reverse transcription. It was shown that 30 μM viomycin was sufficient to protect 50% of G914 from kethoxal modification in 50S ribosomal subunits

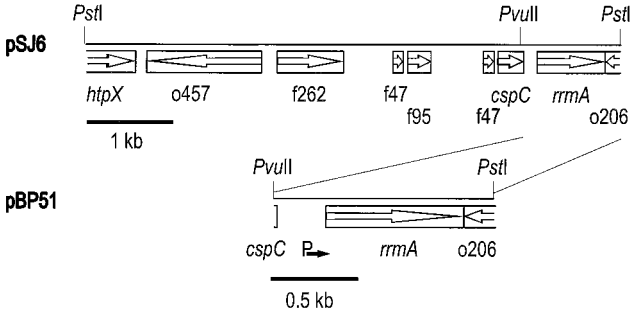

FIG. 4. Gene organization of the chromosomal inserts of plasmids pSJ6 (21) and pBP51. Both plasmids are derivatives of pUC19 (39). Designations of ORFs are from reference 5, where o and f represent different orientations of transcription and the number gives the length of the gene product in amino acids.

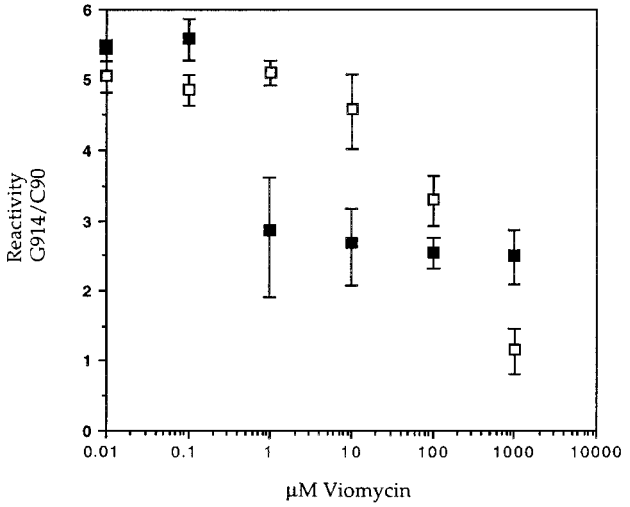

FIG. 5. Viomycin protects G914 100-fold better in strains lacking m[1]G745. Shown is protection by viomycin against kethoxal modification at base G914 in ribosomes containing or lacking m[1]G745. The autoradiogram band intensities were measured with a PhosphorImager and are given as the ratio to the protection of C908, a base which is unaffected by viomycin and kethoxal. Each bar represents at least two independent experiments; in each experiment, the samples were done in triplicate. ■, strain IB103 (*rrmA*); □, strain CP79 (wild type).

containing m[1]G745. In 50S subunits lacking m[1]G745, only 0.3 μM is required to achieve the same degree of protection (Fig. 5). The removal of the m[1]G745 modification from 23S thus increases the protection of G914 by a factor of 100.

m[1]G745 is conserved within gram-negative bacteria. The guanosine at position 745 of the DNA template of 23S rRNA is well conserved within a wide range of organisms (22). To determine if the level of conservation at this position reflects the guanosine or m[1]G745, we analyzed the presence of m[1]G745 in some representative organisms. rRNA was prepared from *Micrococcus luteus* and *Bacillus subtilis* (gram-positive bacteria), from *Pseudomonas aeruginosa* and *E. coli* C600 (gram-negative bacteria), and from *Thermus aquaticus* (*Thermotogales*). All the analyzed organisms have a guanosine in the equivalent position of their respective DNA sequences. The presence of m[1]G745 (or other G · C base-pair-disturbing modification) was determined by reverse transcriptase as described above. The presence of m[1]G745 was shown to be confined to the gram-negative bacteria *E. coli* and *P. aeruginosa* of the bacteria tested (Fig. 6).

DISCUSSION

In this work, we characterized the gene encoding an enzyme that specifically forms m[1]G in *E. coli* 23S rRNA at position 745. *E. coli* IB10 and IB103 lacking this modified nucleotide have previously been isolated (3). The HPLC profiles of hydrolyzed RNA show the presence of m[1]G in 23S rRNA isolated from *E. coli* CP79 (*rrmA*$^+$) but not in 23S rRNA isolated from the mutated derivative strains IB10 or IB103 (*rrmA*). The HPLC profiles of hydrolyzed 16S rRNA and tRNA are identical between the strains. The molar ratio of m[1]G to m[2]A (1 m[2]A per 23S) in 23S rRNA of CP79 is close to 1, indicating the presence of 1 m[1]G per 23S rRNA molecule. The presence of m[1]G in *E. coli* 23S at position 745 is long established (15). The position of the *rrmA*-related m[1]G was determined with reverse transcriptase, which identified a strong stop at positions 746 to 745 as being the only detectable difference between the 23S rRNA isolated from the *rrmA* mutant and 23S rRNA isolated from the corresponding wild type. Taken together, these facts provide strong evidence that the *rrmA*-encoded protein is 23S rRNA m[1]G745 methyltransferase.

The *rrmA* gene encoding 23S rRNA (m[1]G745) methyltransferase was mapped to min 40.9 on the *E. coli* chromosome by Hfr conjugations and P1 transductions. This region contains one previously sequenced gene, *yebH*, that has characteristics of an RNA-modifying enzyme. The *yebH* ORF contains an *S*-adenosylmethionine-binding site and shows significant homology to a gene, *myrA*, encoding resistance to the antibiotic mycinamicin (16), as well as to *yxjB* from *Bacillus subtilis* (8). The *yebH* ORF is 29% identical to *myrA* and 28% identical to *yxjB* at the amino acid level. Plasmid pBP51 (Fig. 4), containing *yebH*, was transformed into GRB1394 (*rrmA*). The pBP51 plasmid was shown to *trans*-complement both the reduction in growth and the 23S m[1]G methylation as assayed by HPLC, thus confirming that *rrmA* is identical to *yebH*.

A convenient way to analyze changes in the surface of the ribosome is by measuring changes in the binding affinity for different ligands, such as tRNA, proteins, or antibiotics. Many antibiotics interact directly or indirectly with the ribosome to block protein synthesis, and even small changes in the binding surface of the ribosome can result in detectable differences in level of resistance to the antibiotic (9). The increased level of resistance to viomycin presented in this work (fourfold higher than the wild-type level) probably does not reflect a specific resistance mechanism but, instead, may be a consequence of the changes on the surface of the 50S subunit that occur when the hydrophobic group m[1]G is removed or may be an indirect effect of the altered levels of loosely coupled 70S ribosomes.

Viomycin is a member of the tuberatinomycin group of antibiotics. The mode of action of the antibiotic is by blocking translocation and confining the peptidyl-tRNA to the ribosomal A site (27). Viomycin protects G914 in 23S rRNA from chemical modification 100-fold better in the mutant lacking m[1]G745 than in the corresponding wild-type strain (Fig. 5). Still, the mutant IB103 is more resistant to the drug than is the wild-type CP79. This apparent contradiction could be ex-

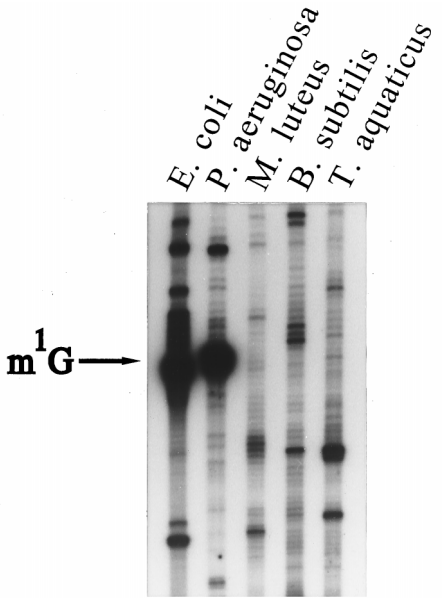

FIG. 6. The modified nucleotide m[1]G is present in purple bacteria. The autoradiogram shows reverse transcription of 23S from various bacterial sources as noted. The position of G745 or its equivalent is marked.

plained by a model where the specific difference between the wild-type and mutant ribosomes is a change of the exact positioning of the viomycin on the 50S subunit rather than a decrease in binding affinity. The chemical protection experiment (Fig. 5) measured only the protection of this particular G914 from chemical modification. Also, the protection of G914 is not necessarily a result of direct interaction of viomycin but could be an indirect effect due to the conformational changes of the ribosome induced by the drug (23).

Strains of *Mycobacterium smegmatis*, resistant to viomycin, had mutations affecting either the 50S or the 30S ribosomal subunits (33, 37). Resistance to viomycin was shown to be determined by 23S rRNA from mutants containing resistant 50S subunits and by 16S rRNA from those with resistant 30S subunits (38). Since strains lacking m^1G745 are resistant to viomycin, as shown above, it was of interest to see if the reverse was true, i.e., if the normal viomycin resistance mechanism involves removal of the methyl group from m^1G745. Strain CP79 was spread on rich-medium plates with 150 μM viomycin, and Vio^r mutants were found at a frequency of 1/1,000. rRNA from 10 Vio^r CP79-derived strains still contained the m^1G745 modification (data not shown). It is likely that the high level of spontaneous Vio^r that we found (1/1,000) is in accordance with this and merely reflects mutations occurring at several positions in both 16S and 23S rRNA, whose structural genes are present in seven copies on the genome, as well as mutations in other factors affecting the overall ribosomal surface in the region interacting with viomycin.

It has been proposed that the general function of modified nucleotides in tRNA and rRNA consists of fine-tuning the translational machinery. In contrast to this hypothesis, the *rrmA* mutant is severely affected by the lack of m^1G745, as is best seen by the 40% reduction in growth rate under nonrestricting conditions. A 40% reduction in growth rate is sufficient for an $rrmA^+$ strain to completely outgrow an isogenic *rrmA* mutant strain in just a few generations. The reduced growth rate may be a consequence of malfunctioning translation. The polysome profile of the *rrmA* mutant strain IB103 reveals sharply reduced levels of loosely bound 70S ribosomes (i.e., 70S ribosomes sensitive to low Mg^{2+} concentrations). It is possible, but not likely, that the polysome distribution and cgr_p are consequences of closely linked mutagenic lesions. The m^1G nucleotide is exposed on the surface of the 50S ribosomal subunit (6, 18) that appears to interact, directly or indirectly, with subunit association, thereby causing decreased binding affinity for the ribosomal 30S subunit. We suggest that the decreased translational velocity (cgr_p) shown in strain IB103 is a consequence of the suboptimal ribosomal subunit association.

ACKNOWLEDGMENTS

We are most grateful to Harry Noller and Glenn Björk, in whose laboratories this work was performed. Kerstin Jacobsson is gratefully acknowledged for excellent technical assistance. We thank Masayori Inouye and Glenn Björk for providing strains and plasmids and Doug Bucklin for helpful comments on the manuscript.

The part of this work that was done in the laboratory of Harry Noller was supported by the National Institutes of Health, the National Science Foundation, the Lucille P. Markey Charitable Trust, and a fellowship to C.G. from the Swedish Natural Science Research Council. B.P. was supported by grants to Glenn Björk from the Swedish Natural Science Research Council (B-BU 2830) and the Swedish Cancer Foundation (project 680).

REFERENCES

1. **Berlyn, M. K. B., K. B. Low, and K. E. Rudd.** 1996. Linkage map of *Escherichia coli* K-12, edition 9, p. 1715–1902. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
2. **Björk, G. R.** 1996. Stable RNA modifications, p. 861–886. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
3. **Björk, G. R., and L. A. Isaksson.** 1970. Isolation of mutants of *Escherichia coli* lacking 5-methyluracil in transfer ribonucleic acid or 1-methylguanine in ribosomal RNA. J. Mol. Biol. **51:**83–100.
4. **Björk, G. R., and K. Kjellin-Stråby.** 1978. General screening procedure for RNA modificationless mutants: isolation of *Escherichia coli* strains with specific defects in RNA methylation. J. Bacteriol. **133:**499–507.
5. **Blattner, F. R., G. Plunkett III, G. F. Mayhew, N. T. Perna, and F. D. Glasner.** 1997. Direct submission DDBJ/EMBL/GenBank databases. Accession no. AE000110 to AE000510.
6. **Brimacombe, R., P. Mitchell, M. Osswald, K. Stade, and D. Bochkariov.** 1993. Clustering of modified nucleotides at the functional center of bacterial ribosomal RNA. FASEB J. **7:**161–167.
7. **Fiil, N., and J. D. Friesen.** 1968. Isolation of "relaxed" mutants of *Escherichia coli*. J. Bacteriol. **95:**729–731.
8. **Fujita, Y.** 1997. Direct submission DDBJ/EMBL/GenBank databases. Accession no. D83026.
9. **Gale, E. F.** 1966. The object of the exercise. Symp. Soc. Gen. Microbiol. **16:**1–21.
10. **Gehrke, C. W., and K. C. Kuo.** 1990. Ribonucleotide analysis by reverse-phase high performance liquid chromatography, p. 3–71. *In* C. W. Gehrke and K. C. Kuo (ed.), Chromatography and modification of nucleosides. A. Analytical methods for major and modified nucleosides. Elsevier Biomedical Press, Amsterdam, The Netherlands.
11. **Gehrke, C. W., K. C. Kuo, R. A. McCune, K. O. Gerhardt, and P. F. Agris.** 1982. Quantitative enzymatic hydrolysis of tRNAs: reversed-phase high-performance liquid chromatography of tRNA nucleosides. J. Chromatogr. **230:**297–308.
12. **Graham, M. Y., and B. Weisblum.** 1979. 23S ribosomal ribonucleic acid of macrolide-producing streptomycetes contains methylated adenine. J. Bacteriol. **137:**1464–1467.
13. **Gutell, R. R., and G. E. Fox.** 1988. A compilation of large subunit RNA sequences presented in a structural format. Nucleic Acids Res. **16**(Suppl.)**:** r175–r269.
14. **Helser, T. L., J. E. Davies, and J. E. Dahlberg.** 1971. Change in methylation of 16S ribosomal RNA associated with mutation to kasugamycin resistance in *Escherichia coli*. Nat. New Biol. **233:**12–14.
15. **Hsuchen, C. C., and D. T. Dubin.** 1980. Methylation patterns of *mycoplasma* transfer and ribosomal ribonucleic acid. J. Bacteriol. **144:**991–998.
16. **Inouye, M., T. Morohoshi, S. Horinouchi, and T. Beppu.** 1994. Cloning and sequences of two macrolide-resistance-encoding genes from mycinamicin-producing *Micromonospora griseorubida*. Gene **141:**39–46.
17. **Isaksson, L. A.** 1973. Partial purification of ribosomal RNA(m^1G)- and rRNA(m^2G)-methylases from *Escherichia coli* and demonstration of some proteins affecting their apparent activity. Biochim. Biophys. Acta **312:**122–133.
18. **Isaksson, L. A.** 1973. Formation in vitro of 1-methylguanine in 23S RNA from *Escherichia coli*. The effects of spermidine and two proteins. Biochim. Biophys. Acta **312:**134–146.
19. **Jensen, K. F., and S. Pedersen.** 1990. Metabolic growth rate control in *Escherichia coli* may be a consequence of subsaturation of the macromolecular biosynthetic apparatus with substrates and catalytic components. Microbiol. Rev. **54:**89–100.
20. **Kagan, R. M., and S. Clarke.** 1994. Widespread occurrence of three sequence motifs in diverse S-adenosylmethionine-dependent methyltransferases suggests a common structure for these enzymes. Arch. Biochem. Biophys. **310:**417–427.
21. **Lee, S. J., A. Xie, W. Jiang, J. P. Etchegaray, P. G. Jones, and M. Inouye.** 1994. Family of the major cold-shock protein, CspA (CS7.4), of *Escherichia coli*, whose members show a high sequence similarity with the eukaryotic Y-box binding proteins. Mol. Microbiol. **11:**833–839.
22. **Maidak, B. L., G. J. Olsen, N. Larsen, R. Overbeek, M. J. McCaughey, and C. R. Woese.** 1996. The Ribosomal Database Project (RDP). Nucleic Acids Res. **24:**82–85.
23. **Martinez, O., D. Vazquez, and J. Modolell.** 1978. Streptomycin- and viomycin-induced conformational changes of ribosomes detected by iodination. FEBS Lett. **87:**21–25.
24. **Miller, J. H.** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Moazed, D., and H. F. Noller.** 1986. Transfer RNA shields specific nucleotides in 16S ribosomal RNA from attack by chemical probes. Cell **47:**985–994.
26. **Moazed, D., and H. F. Noller.** 1987. Chloramphenicol, erythromycin, carbo-

mycin and vernamycin B protect overlapping sites in the peptidyl transferase region of 23S ribosomal RNA. Biochimie **69:**879–884.
27. **Modolell, J., and D. Vazquez.** 1977. The inhibition of ribosomal translocation by viomycin. Eur. J. Biochem. **81:**491–497.
28. **Powers, T., and H. F. Noller.** 1990. Dominant lethal mutations in a conserved loop in 16S rRNA. Proc. Natl. Acad. Sci. USA **87:**1042–1046.
29. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
30. **Schleif, R., W. Hess, S. Finkelstein, and D. Ellis.** 1973. Induction kinetics of the L-arabinose operon of *Escherichia coli*. J. Bacteriol. **115:**9–14.
31. **Singer, M., T. A. Baker, G. Schnitzler, S. W. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:**1–24.
32. **Stern, S., L. M. Changchien, G. R. Craven, and H. F. Noller.** 1988. Interaction of proteins S16, S17 and S20 with 16 S ribosomal RNA. J. Mol. Biol. **200:**291–299.
33. **Taniguchi, H., B. Chang, C. Abe, Y. Nikaido, Y. Mizuguchi, and S. Yoshida.** 1997. Molecular analysis of kanamycin and viomycin resistance in *Mycobacterium smegmatis* by use of the conjugation system. J. Bacteriol. **179:**4795–4801.
34. **Thompson, J., F. Schmidt, and E. Cundliffe.** 1982. Site of action of a ribosomal RNA methylase conferring resistance to thiostrepton. J. Biol. Chem. **257:**7915–7917.
35. **Wikström, P. M., A. S. Byström, and G. R. Björk.** 1988. Non-autogenous control of ribosomal protein synthesis from the *trmD* operon in *Escherichia coli*. J. Mol. Biol. **203:**141–152.
36. **Wrzesinski, J., K. Nurse, A. Bakin, B. G. Lane, and J. Ofengand.** 1995. A dual-specificity pseudouridine synthase: an *Escherichia coli* synthase purified and cloned on the basis of its specificity for Ψ746 in 23S RNA is also specific for Ψ32 in $tRNA^{Phe}$. RNA **1:**437–448.
37. **Yamada, T., K. Masuda, K. Shoji, and M. Hori.** 1972. Analysis of ribosomes from viomycin-sensitive and -resistant strains of *Mycobacterium smegmatis*. J. Bacteriol. **112:**1–6.
38. **Yamada, T., Y. Mizugichi, K. H. Nierhaus, and H. G. Wittmann.** 1978. Resistance to viomycin conferred by RNA of either ribosomal subunit. Nature **275:**460–461.
39. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.