The FtsJ/RrmJ Heat Shock Protein of *Escherichia coli* Is a 23 S Ribosomal RNA Methyltransferase*

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Ribosomal RNAs undergo several nucleotide modifications including methylation. We identify FtsJ, the first encoded protein of the *ftsJ-hflB* heat shock operon, as an *Escherichia coli* methyltransferase of the 23 S rRNA. The methylation reaction requires S-adenosylmethionine as donor of methyl groups, purified FtsJ or a S150 supernatant from an FtsJ-producing strain, and ribosomes from an FtsJ-deficient strain. *In vitro*, FtsJ does not efficiently methylate ribosomes purified from a strain producing FtsJ, suggesting that these ribosomes are already methylated in *vivo* by FtsJ. FtsJ is active on ribosomes and on the 50 S ribosomal subunit, but is inactive on free rRNA, suggesting that its natural substrate is ribosomes or a pre-ribosomal ribonucleoprotein particle. We identified the methylated nucleotide as 2′-O-methyluridine 2552, by reverse phase high performance liquid chromatography analysis, boronate affinity chromatography, and hybridization-protection experiments. In view of its newly established function, FtsJ is renamed RrmJ and its encoding gene, *rrmJ*.

The ribosome is a complex ribonucleoprotein particle that is responsible for translation of messenger RNAs into proteins. In *Escherichia coli*, it is composed of 23, 16, and 5 S ribosomal RNAs and of about 52 proteins. Twenty-one of them assemble with the 16 S rRNA to form the 30 S ribosomal subunit, while the 31 others assemble with the 23 and 5 S rRNA to form the 50 S ribosomal subunit (1). Ribosomal proteins and rRNAs cooperate both in the assembly and in the activity of the ribosome (1). The functional domains of the ribosome include a GTPase center, a peptidyl transferase center, and A-, P-, and E-tRNA binding sites; they involve specific regions of the rRNAs and one or several ribosomal proteins (1). The 16 and 23 S ribosomal RNAs and several ribosomal proteins are methylated at specific sites. The mature 16 and 23 S rRNAs have 10 and 14 methylated nucleotides, respectively (2, 3). The methyl groups are clustered at the functional domains, e.g. the A- and P-tRNA binding sites for 16 S rRNA, and the peptidyly transferase center for 23 S rRNA (4). Most of the modified nucleotides are conserved (5); however, their functions are poorly understood. It has been suggested that methylation could modulate rRNA maturation, affect stability of rRNA structures, or alter translation rates. The nucleotide modifications of rRNAs can modify the susceptibility of ribosomes to antibiotics that target them (6); it is reasonable to expect that the binding of other ligands can be affected as well. In *E. coli*, three 16 S RNA methyltransferases have been identified, RsmA (also known as KsgA) (7), RsmB (2, 8) and RsmC (9). In contrast, little is known about genes involved in 23 S rRNA modifications. The 23 S rRNA displays 23 nucleotide modifications, of which 14 correspond to methylation. Recently, the *rrmJ* gene encoding a 23 S rRNA methyltransferase that forms m1G745 was identified (10). In the present study, we show that the heat shock protein FtsJ methylates the 50 S ribosomal subunit on its 23 S rRNA in *vivo*. *ftsJ* is the upstream gene of a bi-cistronic operon that includes hflB (also known as *ftsH*) (11, 12). Whereas the HflB heat shock protease has been extensively studied, the function of FtsJ had not yet been characterized. In view of its newly established function, FtsJ has been renamed RrmJ and its encoding gene, *rrmJ*.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, and Growth Conditions**—*E. coli* K12 strains C600 (leuB6 thr-1 thi-1 supE44), AR1147 (C600 rrmJ/ftsJ::kan), and C600 (pAR550) were kindly provided by Teru Ogura (University of Kumamoto, Kumamoto, Japan) (11). The *E. coli* B/r strain AD494 (ara leu7967 lacX74 phoA phoR-malF3 F’ [lac+] [lacIq] pro) trxB::kan [DE3] pLysS was purchased from Novagen. Cells were grown in LB broth in the presence of antibiotics, as required (13). Plasmid pAR550 carries the *rrmJ/ftsJ* gene (14). Plasmid pNTRRmJ encodes the complete RrmJ/FtsJ protein fused to a chitin binding domain tag at its N terminus; it was constructed by amplifying the *rrmJ/ftsJ* gene from plasmid pAR550 using primers (5′-GGTTGGTT-GCTTCCATTACGAGGTAAGACGGTTTC-3′) and (5′-GGTTGG-TCTGGATCATTAGGTTGGTGGCGCGC-3′). The resulting product was cut with *SapI* and *PstI* and ligated to the pTYB11 (New England Biolabs, Inc.) *SapI* and *PstI* backbone fragment. The recombinant protein encoded by pINTRRmJ is under the control of a T7 promoter; it is produced in strain AD494 upon addition of isopropyl-β-D-thiogalactopyranoside (IPTG).

**Preparation of S150 and Ribosomes**—S150 supernatants and ribosomes were prepared by mechanical lysis of log phase bacteria in buffer 1 (10 mM Tris, pH 7.4, 10 mM MgCl2, 60 mM NH4Cl, 6 mM 2-mercaptoethanol), followed by ultracentrifugation (15).

1 The abbreviations used are: IPTG, isopropyl-β-D-thiogalactopyranoside; AdaMet, S-adenosylmethionine; CBD, chitin binding domain; Rrm, ribosomal RNA methyltransferase; HPLC, high performance liquid chromatography; Um, 2′-O-methyluridine; MES, 4-morpholineethanesulfonic acid.
Preparation of Ribosomal 50 and 30 S Subunits—Ribosomal subunits were separated by sedimentation through 5–20% sucrose gradients prepared in buffer 2 (buffer 1 supplemented with 0.4 mM NaCl) (18). The pelleted subunits were resuspended in buffer 1 and stored at -70 °C.

Preparation of Ribosomal RNA—rRNAs were prepared from ribosomes by phenol extraction and ethanol precipitation. They were dissolved in 50 mM Tris, pH 7.4, 3 mM Mg(Oac)2 (unless otherwise indicated), 200 mM NH₄Cl, 5 mM dithiothreitol, 2 µM [methyl-³H]AdoMet (80 Ci/mmol for autoradiography experiments, 4 Ci/mmol for trichloroacetic acid precipitations), rRNA or ribosomes, and S₁₅₀ or purified RrmJ/FtsJ, as indicated, and 500 units/ml RNasin (Promega). Incubations were carried out at 37 °C in a total volume of 10 µl. Reactions were terminated by precipitation with cold trichloroacetic acid for kinetic studies (18) or by extraction with phenol saturated with 1% sodium dodecyl sulfate for autoradiographic analysis on polycrylamide gels (18).

Polyacrylamide Gel Electrophoresis of ³H-Labeled RNA—Electrophoresis was performed in nondenaturing polycrylamide gels (3.5% polycrylamide, 0.12% bis(acrylamide)) made up in TBE gel buffer (0.089 M Tris borate, pH 8.3, 2 mM EDTA) (19). The RNA bands were detected by ethidium bromide staining. Gels were incubated for 30 min with the fluorographic reagent Amplify (from Amersham Pharmacia Biotech) and dried before autoradiography.

RESULTS

FtsJ/RrmJ Is a Putative Methyltransferase—The ftsJ gene encodes a putative cytoplasmic alkaline protein of 209 amino acids. The gene is not essential under laboratory conditions (11), and it is transcribed from two promoters, one of which is a heat shock promoter (25). Putative methyltransferases can be predicted (26) by the presence of three motifs that are presumably involved in AdoMet binding (27, 28). The FtsJ sequences VTVTIGKGRIIACDDL, PDVIPDR, and VLANPSSFV (amino acid positions 69–84, 136–142, and 158–167, respectively) are similar to the consensus motifs I, II, and III (27, 28). Putative FtsJ AdoMet binding motifs were previously reported (29); however, the authors did not find motif II, and proposed a different motif I (which is also different from that reported in Ref. 27). FtsJ displays strong similarities with several protein isoaspartate methyltransferases, with the PrmA ribosomal protein methyltransferase and with a putative S-adenosylmethionindependent enzyme from yeast (29–31). Since our results show that FtsJ methylates the 23 S ribosomal RNA, and since the ftsJ-deficient strain AR1147 did not show significant filamentation at 30, 37 and 42 °C (data not shown), we refer to the protein and to its coding gene as RrmJ (for ribosomal RNA methylation) and ftsJ, respectively.

Ribosomal RNA Derived from a FtsJ/RrmJ-deficient Strain Is Methylated by Extracts from a FtsJ/RrmJ-producing Strain—We first checked whether RrmJ catalyzes protein methylation, by incubating supernatants and particulate fractions from a RrmJ-deficient strain and from the RrmJ-overproducing strain in the presence of [methyl-³H]AdoMet. Under appropriate conditions, we observed a ³H-labeled band of molecular mass higher than 200 kDa on SDS-polyacrylamide gels. This band was resistant to prior protease treatment and disappeared if samples were treated with RNase A before electrophoresis, suggesting that RrmJ could be involved in rRNA methylation. We therefore assayed the activity of different fractions on ribosomes. Crude extracts from the RrmJ-overproducing strain (f) and from the RrmJ-deficient strain (d) were prepared by mechanical lysis of bacteria. These extracts were separated into supernatant fractions (S₁₅₀ and S₁₅₀ respectively) and ribosomal fractions (R₁₅₀ and R₁₅₀ respectively), by ultracentrifugation. Incorporation of methyl groups from [methyl-³H]AdoMet into phenol-extracted material was analyzed by polyacrylamide gel electrophoresis (Fig. 1). Incubation of ribosomes from the strain depleted for RrmJ (R₁₅₀) with the S₁₅₀ supernatant from the RrmJ-overproducing strain (S₁₅₀) leads to incorporation of labeled methyl groups in a band that migrates around 18 S RNA. This band (lane 7) could correspond either to 18 S RNA or, as confirmed below, to a degradation product of 23 S RNA (lane 1). This radioactive band disappears if the sample is treated with 10 min with RNase A before loading the gel (data not shown). No significant incorporation of methyl groups into RNA was observed upon incubation of S₁₅₀ with R₁₅₀ (lane 4), S₁₅₀ with R₁₅₀ (lane 5), S₁₅₀ with R₁₅₀ (lane 6), S₁₅₀ or S₁₅₀ alone (lanes 1 and 2, respectively), S₁₅₀ plus S₁₅₀ (lane 3), or R₁₅₀ or R₁₅₀ alone (lanes 8 and 9, respectively). The above experiments were also performed with the wild type strain C6000 and gave results similar to those obtained with the RrmJ-overproducing strain (data not shown). These results show that S₁₅₀, but not S₁₅₀, methylates R₁₅₀. An RNA methyltransferase is thus present in the supernatant of RrmJ-producing strains but not in the RrmJ-deficient strain. This methyltransferase is likely to methylate ribosomes in vivo, since ribosomes from RrmJ-producing strains do not ac-
cept labeled methyl groups in vitro.

RrmJ/FtsJ Purification—We purified RrmJ to confirm its proposed activity in vitro. A chimera protein of RrmJ fused to a chitin-binding domain was purified by affinity chromatography on a chitin affinity column. The CBD is separated from the RrmJ protein by an intein that undergoes self-cleavage by addition of 1,4-dithiothreitol, thus releasing the predicted wild type RrmJ protein from the chitin-bound intein tag (20, 21) (Fig. 2). AD494 pINTRRNJ induced by IPTG expressed a soluble protein of 78 kDa corresponding to the chimera CBD-RrmJ protein (Fig. 2, lanes 2 and 3). The protein released is of 23 kDa, corresponding to the expected molecular mass of RrmJ (lane 6).

RrmJ/FtsJ-dependent Methylation of the 23 S Ribosomal RNA—Ribosomes extracted from the RrmJ-deficient strain were incubated with [methyl-3H]AdoMet, in the absence or presence of purified RrmJ. Incorporation of methyl groups into rRNA was analyzed by trichloroacetic acid precipitation and autoradiography of polyacrylamide gels. RrmJ is able to incorporate 0.8 pmol of methyl groups/pmol of ribosomes in trichloroacetic acid-precipitable material in 20 min, compared with 0.03 pmol of methyl groups/pmol of ribosomes incorporated in the absence of RrmJ (Fig. 3A). After the plate has been attained, addition of more rRNA (22 pmol) leads to an additional methyl incorporation (18 pmol), whereas addition of more enzyme does not produce any effect (Fig. 3A). Analysis of the methylation products on a polyacrylamide gel reveals a major band at the position of 23 S RNA, and a weaker band around 16 S (Fig. 3B, lanes 2–5). When the S150 supernatant

![Fig. 1. Methylation of ribosomal RNA by the S150 supernatant from the RrmJ/FtsJ-overproducing strain. 10 μg of S150 (S) and 3 pmol of ribosomes (R) from the RrmJ-deficient strain (rrmJ::kan) (S150, R1) and from the RrmJ-overproducing strain (carrying pAR550) (S150, R3) were assayed for methylation with [methyl-3H]AdoMet for 30 min at 37 °C, as described under “Experimental Procedures.” Nucleic acids were extracted with phenol and analyzed on a 3.5% polyacrylamide gel. Lane 1, S150; lane 2, S150; lane 3, S150; lane 4, S150; lane 5, S150; lane 6, S150; lane 7, S150; lane 8, R3; lane 9, R3.](http://www.jbc.org/)

![Fig. 2. Purification of RrmJ/FtsJ. Protein samples were separated by sodium dodecyl sulfate-polyacrylamide gels (12%), and stained with Coomassie Brilliant Blue. Lane 1, crude extract from uninduced AD494 pINTRRNJ; lane 2, crude extract from AD494 pINTRRNJ induced by IPTG; lane 3, clarified crude extract from AD494 pINTRRNJ induced cells; lane 4, chitin column flow-through; lane 5, chitin column wash; lane 6, eluted RrmJ after stopping column and inducing a self-cleavage reaction; lane 7, SDS stripping of remaining proteins bound to chitin column (mostly the cleaved intein-chitin binding domain fusion). The molecular size positions are indicated on the left. The position of RrmJ chimera and its processed products are indicated on the right.](http://www.jbc.org/)

...from the RrmJ-overproducing strain is used instead of purified RrmJ, two bands migrating at similar positions are also observed (lane 8). However, the 23 S band is weak, and the 16 S band is strong; it is likely that the 16 S band reflects the action of RNases on 23 S RNA. The in vitro specificity of the methylation catalyzed by RrmJ is demonstrated by the inability of ribosomes from the RrmJ-overproducing strain and from the wild type strain to accept labeled methyl groups from [methyl-3H]AdoMet (lanes 6 and 7, respectively). These results suggest that the in vitro methylation catalyzed by RrmJ is representative of a physiological methylation occurring in vivo. Interestingly, the weak incorporation of labeled methyl groups by ribosomes from the wild type strain (lane 7) suggests that RrmJ methylates these ribosomes in vivo at 37 °C, even in the absence of heat shock (RrmJ is a heat shock protein, which is also expressed from a non-heat shock promoter; Ref. 25).

Several rRNA methyltransferases, such as RsmB (2), function equally well in EDTA as in the presence of magnesium ions whereas others, like RsmC, require magnesium ions for an efficient methylation reaction (9). The RrmJ methyltransferase shows a stringent dependence on magnesium concentration, with a peak centered around 2 mM magnesium, and its activity is severalfold lower in the presence of 10 mM EDTA or in the presence of 10 mM magnesium (data not shown). This result is consistent with the fact that RrmJ is more active on ribosomes rather than free rRNAs as the methylation substrate (see below).

RrmJ/FtsJ Is Active on Ribosomes but Not on Free rRNAs—Some rRNA methyltransferases, such as RsmB (2), methylate rRNA rather than ribosomes, while others, such as RsmC (9), are active on ribosomes but not on free rRNA. We compared the RrmJ-dependent transfer of methyl groups from [methyl-3H]AdoMet to ribosomes and ribosomal RNAs from the RrmJ-deficient strain. Analysis of the methylation reaction on a polyacrylamide gel (Fig. 4) shows that RrmJ actively incorporates methyl groups in the 23 S rRNA of ribosomal particles (lanes 1–4), whereas there is no significant incorporation in the same amount of free rRNA (lanes 5–8). Similar results were obtained by measuring the incorporation of radioactivity from [methyl-3H]AdoMet in trichloroacetic acid-insoluble material (data not shown). Thus, the RrmJ methyltransferase strongly prefers ribosomes to rRNAs as the methyl group acceptor.

RrmJ/FtsJ Methylates the 23 S rRNA of 50 S Ribosomal Subunits—Purified RrmJ catalyzes efficient incorporation of methyl groups into trichloroacetic acid-precipitable material with 50 S ribosomal particle as substrate, whereas no incorporation occurs with 30 S ribosomal particles (Fig. 5). Similarly, the S150 supernatant from the RrmJ-overproducing strain cat-
alyzes incorporation of methyl groups into 50 S ribosomal particles (analyzed by autoradiography of a polyacrylamide gel), but not into 30 S ribosomal particles (not shown).

Identification of the Methylated Nucleotide by HPLC—50 S ribosomal particles were methylated in vitro by purified FtsJ/RrmJ and [methyl-3H]AdoMet at 37 °C. 2 pmol of purified RrmJ were added to an identical reaction mixture after 30 min of incubation (A). 22 pmol of ribosomes from the RrmJ-deficient strain were added to an identical reaction mixture after 30 min of incubation (B). Samples were trichloroacetic acid-precipitated at the times indicated, filtered on Millipore filters, and counted for radioactivity. Analysis on polyacrylamide gel. Mixtures containing variable amounts of RrmJ: 0 ng (lane 1), 6 ng (lane 2), 20 ng (lane 3), 40 ng (lane 4), 100 ng (lanes 5–7), or 10 μg of S150 from the RrmJ-expressing strain (lane 8) and ribosomes (13 pmol) from the RrmJ-deficient strain (lanes 1–5 and 8), from the RrmJ-overproducing strain (lane 6), or from the wild type strain C600 (lane 7) were assayed for methylation for 15 min with [methyl-3H]AdoMet. Samples were removed at the times indicated, extracted with phenol, and analyzed on a 3.5% polyacrylamide gel.

Characterization of the methylated nucleoside by HPLC. Purified RrmJ/FtsJ and [methyl-3H]AdoMet were used to methylate 50 S rRNA in vitro by purified FtsJ/RrmJ and [methyl-3H]AdoMet, as described above. The 3H-methylated 23 S rRNA was phenol-extracted, ethanol-precipitated, and dissolved in distilled water. This [3H]rRNA (0.7 pmol of methyl-3H/pmol) was digested with nuclease P1, snake venom phosphodiesterase I, and bacterial alkaline phosphatase. The resulting nucleoside mixture was analyzed by reverse-phase HPLC, and 5-min elution fractions were collected and counted for 3H radioactivity, as described under “Experimental Procedures.” As shown in Fig. 6, most of the 3H radioactive material was located in fraction 6 corresponding to HPLC retention times between 25 and 30 min. This fraction was rechromatographed on the same HPLC column (1-min elution fractions were collected), and the radioactive peak eluted in the 27–28-min fraction (data not shown). Its elution time is similar to that of 2′O-methyluri-
methylation of ribosomal RNA methylated. This opens the possibility for a stress-dependent. However, a residual ability to accept labeled methyl groups, RrmJ.

FIG. 7. Localization of the site of methylation by RrmJ/FtsJ. 0.6 pmol of methyl-\(^{3}H\)-labeled 23 S RNA, prepared using RrmJ/FtsJ as described in legend to Fig. 6, was hybridized with the indicated oligodeoxyribonucleotide and then digested with RNase T1 (1–10 Sambrook units/pmol of RNA) as described under "Experimental Procedures." Samples were precipitated with trichloroacetic acid (TCA) and counted for radioactivity: no oligomer (○), oligomer 1915 (■), oligomer 2449 (▲), and oligomer 2552 (▲).

dine, Um (27.3 min; data not shown). Furthermore, the radioactive fraction exhibited the same UV absorption spectrum as Um. Since 3-methyluridine (m\(^{3}\)U) co-elutes with Um in our HPLC conditions, and also displays an UV spectrum similar to that of Um (data not shown), we loaded the radioactive fraction onto a boronate affinity column, which retains nucleosides containing an unmodified ribose, like m\(^{3}\)U, but not nucleosides containing 2’-O-methylated ribose, such as Um. The radioactive nucleoside was not retained on the boronate column (data not shown), and could thus be identified as \([^{3}H]Um\).

Localization of the Site of Methylation—E. coli 23 S RNA contains three methylated uridines, located at positions 1915, 2449, and 2552 (3). Uridine 2552 has been characterized as Um, whereas the nature of the methylated uridines at positions 1915 and 2449 has not been determined (3). To determine which uridine is methylated by FtsJ/RrmJ, hybridization-protection studies were conducted using deoxynucleotides complementary to the RNA sequences spanning each of the methylated uridines. As shown in Fig. 7, oligomer 2552 (which spans the 2543–2562 RNA region) strongly protects the site of the \(^{3}\)H-methyl group from RNase T1 digestion, whereas oligomer 1915 (which spans the 1906–1925 RNA region) and oligomer 2249 (which spans the 2240–2259 region) are totally ineffective. These results show that RrmJ/FtsJ is a 23 S RNA methyltransferase, which methylates uridine 2552 at the 2’ OH of the ribose.

DISCUSSION

In the present study, we show that RrmJ methylates the 23 S RNA of the 50 S ribosomal subunit in vitro. The methylation reaction requires S-adenosylmethionine as donor of methyl groups, ribosomes from an rrrmJ-deficient strain, and purified RrmJ or a S150 supernatant from a RrmJ-producing strain. The requirement for ribosomes from a strain deficient in RrmJ as methyl acceptor suggests that the in vitro methylation described in this work reflects in vivo methylation of 23 S ribosomal RNA by RrmJ. The inability of ribosomes from wild type strains to act as substrate for in vitro methylation reactions has been reported by others (15). The fact that ribosomes from the wild type strain grown in normal conditions at 37 °C are poor acceptors of methyl groups in vitro suggests that RrmJ methylates these ribosomes in vivo, even in the absence of heat shock. However, a residual ability to accept labeled methyl groups, compared with an inability of ribosomes from RrmJ-overproducing strains to do so, suggests that they are not completely methylated. This opens the possibility for a stress-dependent methylation of ribosomal RNA in vivo by the heat shock protein RrmJ.

23 S ribosomal RNA is clearly identified as the substrate of RrmJ methyltransferase; purified RrmJ incorporates methyl groups into a band which migrates as 23 S RNA, and 50 S ribosomal particles but not 30 S ribosomal particles can act as methyl acceptors. Consequently, the radioactive band around 16 S RNA observed in the presence of crude extracts probably results from a degradation of 23 S RNA into 16 S RNA. Such degradation of 23 S RNA has frequently been observed (1).

RrmJ methyltransferase is active on ribosomes or ribosomal particles, rather than on free RNA. This property is shared by RsmA and RsmC enzymes (9, 32), and is true of most rRNA-modifying enzymes, with the exception of RsmB (2). RrmJ-mediated methylation requires magnesium; this requirement has also been reported for activity of other rRNA methyltransferases, and appears to be characteristic of reactions involving ribosomes or ribosomal particles (9).

Little is known about the physiological roles of ribosomal RNA methylation, since most experiments have been made in vitro, and since only few rRNA methyltransferases have been identified. Ribosomes reconstituted in vitro with completely unmodified rRNA are active, suggesting a minor role of RNA methylation in ribosome function. In contrast, mutations of 23 S ribosomal RNAs at several methyl-modified regions have dramatic effects on cell viability, reflecting the importance of these sites for proper ribosome function in vivo. FtsJ catalyzes a 2’-O-ribose methylation of Um-2552. This is a universally conserved nucleotide within the peptidyltransferase center of domain V of the large subunit rRNA (33). Um-2552 is adjacent to bases protected by tRNA bound in the A site (33), and mutations in one of these bases, U-2555, affects translational accuracy (34). The growth properties, translation properties, polysomes profile and antibiotic sensitivity of the ftsJ-deficient strain are under investigation.

In E. coli, the rluA, rluC, and rluD (35) genes encode pseudouridine synthase, which modifies 23 S rRNA. Curiously, an allele of rluD is a suppressor of a thermosensitive mutation in hflB, the downstream gene of the rrmJ-hflB operon. hflB encodes an ATP-dependent protease involved in the degradation of s32 and other subunits. These observations raise the possibility that the rrmJ-hflB operon might encode two proteins involved in ribosomal regulation.

RrmJ homologues are found in eubacteria, archaea, and eu-karyotes. However, among 24 completely sequenced microorganisms, only 9 of them encode putative RrmJ homologues. If the rRNA methylation mediated by RrmJ is conserved, it could be performed by methylases lacking obvious sequence similarities with RrmJ. Surprisingly, the JM 23 human protein encoded by chromosome X has 35% identity (52% conserved) over 192 amino acids with RrmJ, suggesting that, in at least certain higher organisms, rRNA methylation could be performed by an enzyme closely related to the E. coli RrmJ.

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