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U2552 methylation at the ribosomal A-site is a negative modulator of translational accuracy

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Abstract

We have recently identified RrmJ, the first encoded protein of the *rrmJ-ftsH* heat shock operon, as being the Um_{2552} methyltransferase of 23S rRNA, and reported that *rrmJ*-deficient strains exhibit growth defects, reduced translation rates and reduced stability of 70S ribosomes. U2552 is an ubiquitously methylated residue. It belongs to the A loop of 23S RNA which is an essential component of the ribosome peptidyltransferase centre and interacts directly with aminoacyl(A)-site tRNA. In the present study, we show that a lack of U2552 methylation, obtained in *rrmJ*-deficient mutants, results in a decrease in programmed +1 and −1 translational frameshifing and a decrease in readthrough of UAA and UGA stop codons. The increased translational accuracy of *rrmJ*-deficient strains suggests that the interaction between aminoacyl-tRNA and U2552 is important for selection of the correct tRNA at the ribosomal A site, and supports the idea that translational accuracy in vivo is optimal rather than maximal, thus pointing to the participation of recoding events in the normal cell physiology.

Keywords: *Escherichia coli*; *rrmJ*/*ftsJ*; 23S RNA; Methyltransferase; Frameshift; Readthrough

1. Introduction

The ribosome is a complex ribonucleoprotein particle that is responsible for translation of messenger RNAs into proteins. In *Escherichia coli*, it is composed of 23S, 16S, and 5S ribosomal RNAs and about 52 proteins. Twenty-one of these proteins assemble with the 16S rRNA to form the 30S ribosomal subunit while the other 31 assemble with the 23S and 5S rRNA to form the 50S ribosomal subunit (Noller, 1991). The functional domains of the ribosome include a GTPase centre, a peptidyl transferase centre, and A-, P-, and E-tRNA binding sites. They involve specific regions of the rRNAs and one or several ribosomal proteins (Noller, 1991; Noller et al., 2001; Yusupov et al., 2001).

The 16S and 23S ribosomal RNAs, and several ribosomal proteins are methylated at specific sites. The mature *E. coli* 16S and 23S rRNAs have 10 and 14 methylated nucleotides, respectively (Tscherne et al., 1999a; Smith et al., 1992). The methyl groups are clustered around the functional domains, e.g., the A- and P-tRNA binding sites for 16S rRNA, and the peptidyltransferase centre for 23S rRNA. Most of the modified nucleotides are conserved (Moore and Steitz, 2002). In *E. coli*, three 16S RNA methyltransferases, RsmA (van Buul and van Knippenberg, 1985), RsmB (Tscherne et al., 1999a) and RsmC (Tscherne et al., 1999b) and five 23S RNA methyltransferases, RrmA (Gustafsson and Persson, 1998), RlmB (Lovgren and Wilkstrom, 2001), RumA (Agarwalla et al., 2002), RumB (Madsen et al., 2003) and RrmJ (Caldas et al., 2000a,b; Bugl et al., 2000) have been described: The *rrmA* gene encodes a 23S rRNA methyltransferase that forms m^1G745, RlmB is essential for the formation of Gm2251, RumA is the m^5U1939 methyltransferase, RumB is the m^5U747 methyltransferase and RrmJ is the methyltransferase that catalyzes the 2′-*O*-methyl ribose modification at position U2552 (Caldas et al., 2000a).

Abbreviations: Ψ, Pseudouridine.

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Um2552 corresponds to one of the few highly conserved methylated nucleotides found in the otherwise minimally modified mitochondrial rRNA from *Saccharomyces cerevisiae* (Sirum-Connolly et al., 1995), which suggests that it plays an important role in ribosome function. It belongs to the A loop, which is an essential 23S RNA component of the peptidyl transferase centre that directly interacts with the aminoacyl end of the aminoacyl (A)-site tRNA after release of elongation factor EF-Tu (Moazed and Noller, 1989; Kim and Green, 1999). Its neighbouring residues Ψ2555 and G2553 are protected from chemical modification by the aminoacyl moiety and the terminal adenine of aminoacyl-tRNA, respectively (Moazed and Noller, 1989). Moreover U2552 methylation modifies the A-loop fold and in particular the dynamics and positions of residues C2556 and U2555 (Blanchard and Puglisi, 2001).

RrmJ is active on ribosomes and on the 50S ribosomal subunit, but is inactive on free rRNA, suggesting that its natural substrate is ribosomes (Caldas et al., 2000a; Hager et al., 2002). *RrmJ*-deficient strains have a slow growth phenotype and display several translational defects such as an increased proportion of free 50S and 30S ribosomal subunits, and a decrease in protein synthesis activity in vitro (Caldas et al., 2000a,b; Bugl et al., 2000). While *E. coli* cells have only one RrmJ homologue, eukaryotic cells may have several (Pintard et al., 2002). In the present study, we show that a lack of U2552 methylation results in a decrease in frameshifting and readthrough in vivo. Translational accuracy is crucial for the expression of genetic information. During mRNA translation, the ribosome selects the correct aminoacyl-tRNA in the form of a ternary complex with elongation factor Tu and GTP on the basis of the match between codon and anti-codon triplets. Aminoacyl-tRNA selection involves an initial selection step followed by a proofreading step (accomodation) occurring after GTP hydrolysis but before peptide bond formation, during which the aminoacyl end of aa-tRNA is free to move into the peptidyltransferase centre on the 50S subunit (Rodnina and Wintermeyer, 2001; Moore and Steitz, 2002; Stahl et al., 2002). Three groups of structural elements of the ribosome influence the fidelity of protein synthesis. The first group includes those regions of 16S RNA and proteins of the small ribosomal subunit that form the decoding region, involved in sensing the structure of the codon–anticodon complex and in creating the conformational signal of codon recognition (Ramakrishnan, 2002; Ogle et al., 2003). The second group includes parts of rRNA at the subunit interface that mediate transmission of the conformational signal to the 50S subunit, and the third group comprises regions of the 50S subunit that receive and convert the signal by accelerating EF-Tu GTPase activation and aa-tRNA accommodation (Saarma and Remme, 1992; Rodnina and Wintermeyer, 2001). Mutations in these elements influence translational accuracy (Rodnina and Wintermeyer, 2001; Moore and Steitz, 2002; Stahl et al., 2002). Mutations in ribosomal proteins S12, S17, L6, or in 23S RNA at position G2583 or in the 2660 loop increase accuracy, whereas mutations in ribosomal proteins S4, S5, L7/L12, EF-Tu, Release factors 1 and 2, in 16S RNA at positions 530, 960, 1400, 1490 or in 23S RNA at position 2555 decrease the accuracy of protein synthesis. The increased translational accuracy of an unmethylated U2552 strain suggests that the interaction between aminoacyl-tRNA and this 23S RNA residue is important for selection of the correct tRNA at the ribosomal A site.

2. Materials and methods

2.1. Bacterial strains, plasmids, growth conditions

The *E. coli* K12 strain JS219 (hsdR2, mcrB1, araD139, Δ(araABC-leu)7679, ΔlacX74, galU, galK, rpsL, thi, malP::lacI^q) and its *rrmJ*-deficient derivative OFB5844 were used for this study (Caldas et al., 2000a; Rettberg et al., 1999). OFB5844 was constructed by transduction of *rrmJ*::*kan* from AR1147 (C600 *rrmJ*::*kan*) to JS219. The series of plasmids containing signals for programmed frameshifting or nonsense mutations in the 5′ end of the lacZ coding region were constructed using the pOFX302 vector (Rettberg et al., 1999). The bicistronic reporter plasmid is an ampicillin-resistant plasmid derived from pBR322 in which gene 10 of bacteriophage T7 is inserted upstream of lacZ. Both genes are under the control of a unique *ptac* promoter. At their junctions, 2 unique restriction sites, *Hin*dIII and *Apa*I, were used for cloning oligonucleotides containing the 5 different inserts. In the "in phase" *lacZ* plasmid, pOFX302-631, the termination codon of gene 10 has been deleted, and gene 10 and *lacZ* are in phase (this represents our 100% translation control). In the +1 frameshift plasmid, pOFX302-330, the RF2 +1 frameshift window (sequence inserted between the *Hin*dIII and *Apa*I sites: AGC TTC AGG GGG TAT CTTT GAG CCT GTA GGC C…; the frameshift site is underlined; see Weiss et al., 1988) has been cloned before the end of the gene 10 termination codon, with *lacZ* downstream of the frameshift window, in the +1 reading frame, so that β-galactosidase expression is a measure of +1 frameshift efficiency. In the −1 frameshift plasmid, pOFX302-627, the dnaX −1 frameshift window (see Fig. 1 in Larsen et al., 1997) has been cloned so that β-galactosidase is a measure of −1 frameshifting. In the pOFX302-947 plasmid, an UAA termination codon was introduced between gene 10 and *lacZ* so that β-galactosidase expression is a measure of UAA readthrough. The similar pOFX302-948 plasmid allows quantitation of UGA readthrough. Bacteria were grown in LB medium (Miller, 1972) for β-galactosidase activity measurements or Mops medium (Neidhardt et al., 1974) supplemented with all amino acids (50 μg/ml each) except methionine for protein labelling. Ampicillin (50 μg/ml) and kanamycin (30 μg/ml) were added when necessary.