

Modification of 16S ribosomal RNA by the KsgA methyltransferase restructures the 30S subunit to optimize ribosome function

HASAN DEMIRCI,^{1,5} FRANK MURPHY IV,^{2,5} RICCARDO BELARDINELLI,³ ANN C. KELLEY,⁴
V. RAMAKRISHNAN,⁴ STEVEN T. GREGORY,¹ ALBERT E. DAHLBERG,¹ and GERWALD JOGL^{1,6}

¹Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island 02912, USA

²NE-CAT/Cornell University, Argonne, Illinois 60439, USA

³Laboratory of Genetics, Department of Biology, Molecular Cell Biology and Animal Biology, University of Camerino, Camerino (MC) 62032, Italy

⁴Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge, Cambridge CB2 0QH, United Kingdom

ABSTRACT

All organisms incorporate post-transcriptional modifications into ribosomal RNA, influencing ribosome assembly and function in ways that are poorly understood. The most highly conserved modification is the dimethylation of two adenosines near the 3' end of the small subunit rRNA. Lack of these methylations due to deficiency in the KsgA methyltransferase stimulates translational errors during both the initiation and elongation phases of protein synthesis and confers resistance to the antibiotic kasugamycin. Here, we present the X-ray crystal structure of the *Thermus thermophilus* 30S ribosomal subunit lacking these dimethylations. Our data indicate that the KsgA-directed methylations facilitate structural rearrangements in order to establish a functionally optimum subunit conformation during the final stages of ribosome assembly.

Keywords: ribosome; rRNA modification; RNA structure; *Thermus*; decoding; antibiotic resistance

INTRODUCTION

All bacteria contain post-transcriptional modifications of their rRNAs that are thought to fine tune ribosome assembly and function. Despite much effort exerted by a number of laboratories over many years, it remains to be determined precisely how rRNA modifications influence ribosome structure, and in turn, how such structural effects manifest themselves biologically. One of the most thoroughly examined rRNA modifications is the N⁶,N⁶-dimethylation of A1518 and A1519 (*Escherichia coli* numbering), in the GGAA tetraloop of 16S rRNA helix 45 (Fig. 1). Both the modification (Van Knippenberg et al. 1984; McCloskey and Rozenski 2005) and the sequence (Woese et al. 1990) of this loop appear to be universally conserved or nearly so, indicative of an important physiological role for this modified structure.

Methylation of both bases is catalyzed by a single methyltransferase, KsgA, at a late stage of 30S subunit assembly (Thammana and Held 1974). Lack of methylation due to *ksgA* mutations confers resistance to the antibiotic kasugamycin (Helser et al. 1971), increases decoding errors during elongation (van Buul et al. 1984), and enhances initiation from non-AUG codons (O'Connor et al. 1997). This pleiotropic phenotype suggests a propagation of structural effects beyond helix 45 and into both the A and P sites of the ribosome.

KsgA and initiation factor 3 (IF3) compete for overlapping binding sites on the 30S subunit, preventing incompletely assembled 30S subunits from engaging in translation initiation prior to methylation by KsgA (Xu et al. 2008). Further, *ksgA* mutants exhibit cold-sensitive 30S subunit assembly and 16S rRNA processing defects (Connolly et al. 2008). These observations implicate KsgA methylation as a checkpoint for 30S subunit assembly. While a role for KsgA in 30S ribosomal subunit assembly is provided by this model, the structural basis for the effects of methylation deficiency on the subsequent events of protein synthesis have not been determined.

To define the role of these conserved modifications in ribosome structure and function, we determined the X-ray

⁵These authors contributed equally to this work.

⁶**Reprint requests to:** Gerwald Jogl, Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island 02912, USA; e-mail: Gerwald_Jogl@brown.edu; fax: (401) 863-6114.

Article published online ahead of print. Article and publication date are at <http://www.rnajournal.org/cgi/doi/10.1261/rna.2357210>.

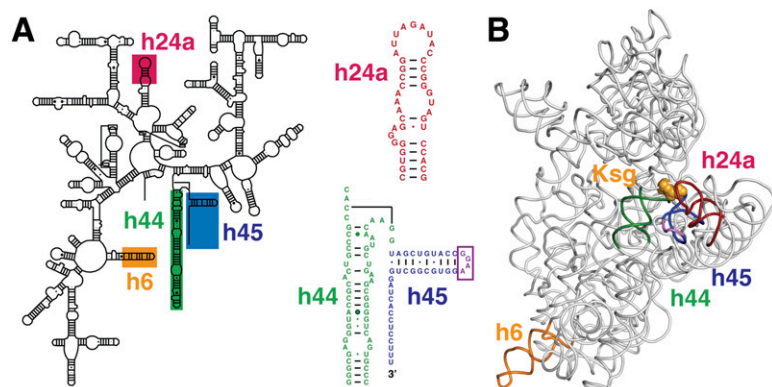


FIGURE 1. Position of the modified nucleotides A1518 and A1519 in the 30S ribosomal subunit. (A) Secondary structure of *T. thermophilus* 16S rRNA (Cannone et al. 2002), indicating positions and sequences of helices 6, 24a, 44, and 45 that show structural changes in the unmethylated 30S subunit. (B) Positions of helices 6, 24a, 44, and 45 in the crystal structure of the *T. thermophilus* 30S subunit (Wimberly et al. 2000). Ribosomal proteins in panel B are omitted for clarity.

crystal structure of the 30S subunit from a *ksgA* deletion mutant of *Thermus thermophilus* HB8 to 3.7-Å resolution. Our structure indicates that methylation facilitates the formation of a packing interaction between helix 45 and helix 44 in the vicinity of the decoding site. Loss of this packing interaction perturbs surrounding rRNA structure in both the A and P sites of the ribosome, providing a structural basis for the pleiotropic phenotype of *ksgA* mutants. We conclude that the KsgA methyltransferase acts to establish an active conformation of the 30S ribosomal subunit that is optimized to participate in protein synthesis.

RESULTS AND DISCUSSION

Crystallization of 30S subunits from a *ksgA* mutant

We previously described a mutant of *T. thermophilus* HB8 in which the *ksgA* gene had been deleted and replaced with a kanamycin-resistance gene (Demirci et al. 2009). 30S subunits from this mutant (hereafter referred to as “unmethylated” subunits) were prepared and crystallized as described by others (Wimberly et al. 2000; Clemons et al. 2001). Unmethylated 30S subunits produced tetragonal crystals in the same space group, $P4_12_12$, that are obtained with fully methylated subunits from wild-type *T. thermophilus*, but crystallized slowly as small needles over a period of 1 yr, rather than in the 2 mo normally required for 30S subunits. Crystallization of unmethylated subunits was completely inhibited by kasugamycin, suggesting that *T. thermophilus* *ksgA* 30S subunits retain the ability to bind kasugamycin, as previously observed with an *E. coli* *ksgA* mutant (Schuwirth et al. 2006).

Slow crystallization suggests the existence of structural changes at sites of crystal contacts. In one such contact, 16S rRNA helix 6 (the so-called “spur”) binds to the P site of

a symmetry-related subunit in a configuration that closely resembles the binding configuration of peptidyl-tRNA (Wimberly et al. 2000). Kasugamycin destabilizes fMet-tRNA^{fMet} binding to the P site in 30S initiation complexes (Poldermans et al. 1979) and could inhibit crystallization by destabilizing the binding of helix 6 to the P site in an analogous manner. Thus, the crystallization behavior of the *ksgA* 30S subunit likely reflects physiologically relevant changes occurring in and around the ribosomal P site.

Effect of methylation on helix 45 loop conformation

Electron density in the vicinity of helix 45 was of sufficient quality to allow

rRNA backbone phosphates and base positions to be clearly distinguished (Fig. 2A). Significant structural changes resulting from lack of methylation are seen in the loop of helix 45 (Fig. 2B,C, violet). This highly conserved GGAA loop is a member of the GNRA tetraloop family that exhibits a fold characterized by a sheared base pair between the first (G1516) and fourth (A1519) bases, with the second (G1517), third (A1518), and fourth (A1519) bases stacking on the 3' side of the loop (Heus and Pardi 1991; Jucker et al. 1996). The unmethylated tetraloop in the context of the 30S subunit closely follows the canonical GNRA fold (Fig. 2D). However, in fully methylated (wild type) 30S subunits (Wimberly et al. 2000) the hydrogen bond between the N2 of G1516 and the N7 of A1519, seen in canonical GNRA tetraloops, is absent, and there is a widening of the loop (Fig. 2C, gray).

These observations are consistent with previous studies demonstrating a thermodynamic destabilization of helix 45 by dimethylation of A1518 and A1519 (Thammana and Cantor 1978; Van Charldorp et al. 1981; Heus et al. 1990; Rife et al. 1998). While an additional methylation at the N2 of G1516 is found in helix 45 of some bacteria but is absent from *T. thermophilus* (Guymon et al. 2006), it does not alter loop thermal stability or interfere with hydrogen bonding (Rife et al. 1998).

Loss of helix packing interactions

In ribosomes from wild-type cells, there is extensive contact between the helix 45 tetraloop and helix 44, near the decoding site (Wimberly et al. 2000). This interaction is distinct from the GNRA tetraloop–receptor interaction observed in other RNA molecules (Cate et al. 1996). Lack of helix 45 methylation in ribosomes from the *ksgA* mutant eliminates all of these hydrogen-bonding interactions (Fig. 2E,F).

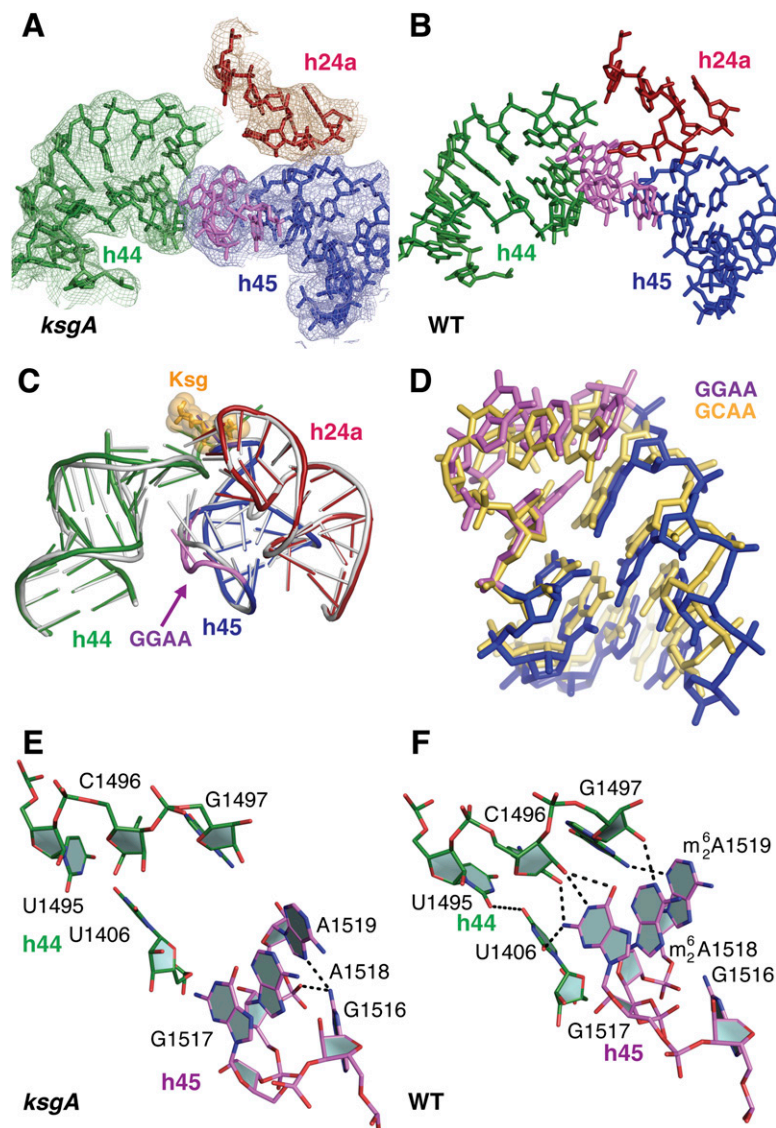


FIGURE 2. Structural changes in the unmethylated 30S subunit. (A) Final σ_A -weighted $2mF_o - DF_c$ electron density in the *ksgA* mutant 30S subunit crystal structure. (Red) 16S rRNA helix 24a (h24a); (green) helix 44 (h44); (blue) helix 45 (h45); (violet) the GGAA tetraloop of helix 45. (B) Contacts between helix 45 and helices 24a and 44. Helices are colored as in A. (C) Bending of the GGAA tetraloop in the unmethylated subunit (violet) is visible. The wild-type 30S subunit structure (PDB entry 1J5E) (Wimberly et al. 2000) is superimposed (gray). Kasugamycin (Ksg, orange spheres) from PDB entry 1VS5 (Schuwirth et al. 2006) is superimposed. (D) Comparison of the helix 45 tetraloop with a GCAA tetraloop as observed in PDB entry 1ZIIH (Jucker et al. 1996). (E,F) The *ksgA* mutation prevents the formation of a hydrogen-bonding network between helices 45 and 44. The helix 45–44 interface in the *ksgA* mutant (e.g., 30S subunits prior to methylation) (E) and in the wild-type, fully methylated 30S subunit (F). Panels E and F are from identical viewpoints.

In the unmethylated 30S subunit, the absence of N^6,N^6 -dimethylation generates a tighter loop structure, with A1519 positioned closer to G1516, establishing the N7–N2 hydrogen bond found in canonical GNRA tetraloops (Fig. 2C). The loop bends significantly, precluding the hydrogen-bonding interactions with helix 44 (Fig. 2C). The N6 atoms of A1518 and A1519 are moved by about 5 Å and 4 Å, respectively, and two hydrogen bonds, between the N1 of

G1517 and the 2'-OH of C1496 and between the N2 of G1517 and the O2 of U1406 are absent as these atoms are 8 Å and 7 Å apart, respectively. Also, hydrogen bonds between the N1 of A1518 and the 2'-OH of G1497, and between N3 of A1519 and N2 of G1497, are absent (Fig. 2E,F). Thus, these data indicate that the initial structural role of N^6,N^6 -dimethylation of A1518 and A1519 is to open up the G1516–A1519 base pair and widen the loop, and in so doing, facilitate the subsequent formation of a hydrogen-bonding network that stabilizes the packing interactions between helices 44 and 45.

Structural basis for the *ksgA* mutant phenotype

In the absence of this hydrogen-bonding network, small conformational differences are noted in the surrounding structure that may account for resistance to kasugamycin (Fig. 3A,B). In helix 24a, U793 is in the space normally occupied by the methylated adenosines, and the two adjacent bases, A792 and A794, show small shifts. These bases comprise part of the kasugamycin binding site (Schluenzen et al. 2006; Schuwirth et al. 2006), and their movement is consistent with the finding that a base substitution, A794G, confers kasugamycin resistance (Vila-Sanjurjo et al. 1999). Thus, as previously predicted (Schluenzen et al. 2006; Schuwirth et al. 2006), kasugamycin resistance due to lack of methylation most likely results from indirect effects on the conformation of helix 24a in the vicinity of bases directly involved in kasugamycin binding.

The absence of the helix 44–45 packing interaction also influences the position of A1492 and A1493, while a shift of up to 6 Å of the 30S subunit head causes a repositioning of C1054 (Fig. 3C).

These three bases participate directly in codon recognition (Ogle et al. 2001), suggesting a basis for the error-prone phenotype of *ksgA* mutants. Lack of methylation might also influence decoding by perturbing the equilibrium of the global conformational change that occurs upon cognate codon recognition (Ogle et al. 2002). This involves the formation of contacts between ribosomal protein S12 and the side of helix 44 opposite the helix 45 packing site (Fig. 3C).

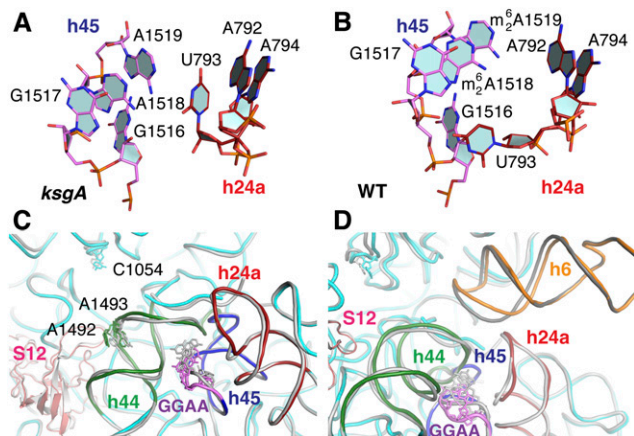


FIGURE 3. Structural readjustments of helices adjacent to the unmethylated GGAA tetraloop. Movement of A793 in the kasugamycin-binding site in unmethylated (A) and fully methylated (Schuwirth et al. 2006) (B) 30S subunits. (C) Close-up view illustrating shifts of A1492, A1493, and C1054 in the decoding site of the unmethylated 30S (cyan) in comparison with the wild-type (gray) subunit (PDB 1J5E) (Wimberly et al. 2000), colored as in Figure 2B. (D) Close-up view illustrating the shift in position of 16S rRNA helix 6 of the unmethylated 30S subunit (orange) in the P site of an adjacent 30S subunit.

If the loss of the helix 44–45 packing influences the dynamics of this contact, it could reduce the thermodynamic barrier to domain closure and increase incorporation of near-cognate aminoacyl-tRNAs in the A site. We also observed a positional shift of the helix 6 spur of an adjacent 30S subunit in the P site (Fig. 3D). To the extent that this crystal contact mimics P-site tRNA binding (Wimberly et al. 2000), this positional shift suggests that distortions in the P site may be the basis for the observed increase in initiation from non-AUG codons (O'Connor et al. 1997). This most likely is the result of changes in helix 24a.

Recognition of 30S subunits by the KsgA methyltransferase

Although the *in vivo* substrate for KsgA is not known, methylation occurs at a late stage of 30S subunit assembly (Thammana and Held 1974), subsequent to incorporation of almost the full complement of ribosomal proteins, but prior to the establishment of a final active 30S subunit conformation (Desai and Rife 2006). Ribosomal protein S21 is inhibitory to methylation by KsgA, although *T. thermophilus* lacks this protein (Henne et al. 2004). The conformation suitable for methylation can be mimicked by reducing the concentration of either magnesium (Demirci et al. 2009) or monovalent cation (Zamir et al. 1969; Desai and Rife 2006). The latter condition induces altered reactivity of 16S rRNA to base-specific chemical probes (Moazed et al. 1986) and also causes the dimethylated adenosines A1518 and A1519 to become accessible to antibodies (Thammana and Cantor 1978). Thus, KsgA

would appear to recognize a nonnative conformation of the 30S subunit that can in some way be mimicked by conditions that perturb or destabilize ribosome structure. While the checkpoint model proposes that KsgA prevents 30S subunits from entering the translating pool prior to final activation (Xu et al. 2008), the structure described here suggests that methylation by KsgA plays an additional direct role in establishing a fully active 30S subunit conformation.

The importance of packing interactions between helices 44 and 45 has been suggested previously by mutagenesis experiments (Vila-Sanjurjo and Dahlberg 2001), but it is the structural data presented here that resolve the long-standing paradox as to why a GGAA tetraloop sequence is so highly conserved in helix 45 while its fold is incompatible with optimum ribosome function. Studies using oligoribonucleotide analogs of helix 45 indicate that methylation influences loop thermal stability (Van Charldorp et al. 1981; Heus et al. 1990; Rife et al. 1998) and fold (Rife and Moore 1998). Thermodynamically stable GNRA tetraloops serve as nucleating sites for RNA folding (Heus and Pardi 1991; Jucker et al. 1996), and this stabilization may be utilized to prevent misfolding of the terminal helix of 16S rRNA during transcription. Subsequent destabilization and distortion of the tetraloop by methylation would then facilitate the formation of a more stable and functional tertiary structure in the final stages of 30S subunit assembly.

MATERIALS AND METHODS

30S ribosomal subunits from the *Thermus thermophilus* HB8 *ksgA* deletion mutant (Demirci et al. 2009) were purified, crystallized,

TABLE 1. Data collection and refinement statistics

	$\Delta ksgA^a$
Data collection	
Space group	P4 ₁ 2 ₁ 2
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	402.1, 402.1, 173.3
α , β , γ (°)	90, 90, 90
Resolution (Å)	30–3.7 (3.76–3.70)
R_{merge}	0.13 (0.59)
$I/\sigma I$	9.7 (1.8)
Completeness (%)	98.2 (97.3)
Redundancy	3.2 (3.1)
Refinement	
Resolution (Å)	30–3.7 (3.73–3.70)
No. of reflections	140,210 (3685)
$R_{\text{work}}/R_{\text{free}}$	0.173/0.231(0.269/0.324)
No. of atoms	51,783
<i>B</i> -factors	127.00
RMS deviations	
Bond lengths (Å)	0.005
Bond angles (°)	0.759

Highest resolution shell is shown in parentheses.

^aOne crystal was used for this data set.

and cryoprotected essentially as described (Clemons et al. 2001). Diffraction data were collected from a single crystal at beamline ID-24-C at the Advanced Photon Source in Argonne, Illinois. Diffraction data were processed with the HKL2000 package (Table 1; Otwinowski and Minor 1997). Coordinates from the wild-type 30S subunit structure (PDB entry 1J5E) (Wimberly et al. 2000) were used for initial rigid-body refinement with PHENIX (Adams et al. 2002). After simulated-annealing refinement, individual coordinates, two group B factors per residue, and TLS parameters were refined against the 3.7-Å data set. Potential positions of magnesium or potassium ions were compared with those in a high-resolution 2.5-Å 30S subunit structure (PDB entry 2VQE) (Kurata et al. 2008) in Coot (Emsley and Cowtan 2004), and positions with strong difference density were retained. All figures were produced with PyMOL (DeLano 2002). Coordinates and structure factors have been deposited in the Protein Data Bank under accession number 3OTO.

ACKNOWLEDGMENTS

This work was supported by grants GM19756 and GM19756-37S1 from the National Institutes of Health to A.E.D. We thank Aiko and Kenji for numerous discussions; Jason Rife, Gloria Culver, Stephen Douthwaite, Jennifer Carr, Anton Vila-Sanjurjo, and James Dahlberg for critical reading of the manuscript; Michael Bender for helpful discussions; and Bruce Fitzgerald and Rick Cole for excellent technical assistance. The authors dedicate this paper to the memory of Warren DeLano and Peter Van Knippenberg.

Received July 9, 2010; accepted September 16, 2010.

REFERENCES

- Adams PD, Grosse-Kunstleve RW, Hung LW, Ioerger TR, McCoy AJ, Moriarty NW, Read RJ, Sacchettini JC, Sauter NK, Terwilliger TC. 2002. PHENIX: Building new software for automated crystallographic structure determination. *Acta Crystallogr D Biol Crystallogr* **58**: 1948–1954.
- Cannone JJ, Subramanian S, Schnare MN, Collett JR, D'Souza LM, Du Y, Feng B, Lin N, Madabusi LV, Müller KM, et al. 2002. The comparative RNA web (CRW) site: An online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. *BMC Bioinformatics* **3**: 2. doi: 10.1186/1471-2105-3-2.
- Cate JH, Gooding AR, Podell E, Zhou K, Golden BL, Szewczak AA, Kundrot CE, Cech TR, Doudna JA. 1996. RNA tertiary structure mediation by adenosine platforms. *Science* **273**: 1696–1699.
- Clemons WM Jr, Brodersen DE, McCutcheon JP, May JL, Carter AP, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V. 2001. Crystal structure of the 30 S ribosomal subunit from *Thermus thermophilus*: Purification, crystallization and structure determination. *J Mol Biol* **310**: 827–843.
- Connolly K, Rife JP, Culver G. 2008. Mechanistic insight into the ribosome biogenesis functions of the ancient protein KsgA. *Mol Microbiol* **70**: 1062–1075.
- DeLano WL. 2002. *The PyMOL Molecular Graphics System*. DeLano Scientific, San Carlos, CA.
- Demirci H, Belardinelli R, Seri E, Gregory ST, Gualerzi C, Dahlberg AE, Jögl G. 2009. Structural rearrangements in the active site of the *Thermus thermophilus* 16S rRNA methyltransferase KsgA in a binary complex with 5'-methylthioadenosine. *J Mol Biol* **388**: 271–282.
- Desai PM, Rife JP. 2006. The adenosine dimethyltransferase KsgA recognizes a specific conformational state of the 30S ribosomal subunit. *Arch Biochem Biophys* **449**: 57–63.
- Emsley P, Cowtan K. 2004. Coot: Model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* **60**: 2126–2132.
- Guymon R, Pomerantz SC, Crain PF, McCloskey JA. 2006. Influence of phylogeny on posttranscriptional modification of rRNA in thermophilic prokaryotes: The complete modification map of 16S rRNA of *Thermus thermophilus*. *Biochemistry* **45**: 4888–4899.
- Helser TL, Davies JE, Dahlberg JE. 1971. Change in methylation of 16S ribosomal RNA associated with mutation to kasugamycin resistance in *Escherichia coli*. *Nat New Biol* **233**: 12–14.
- Henne A, Brüggemann H, Raasch C, Wiezer A, Hartsch T, Liesegang H, Johann A, Lienard T, Gohl O, Martinez-Arias R, et al. 2004. The genome sequence of the extreme thermophile *Thermus thermophilus*. *Nat Biotechnol* **22**: 547–553.
- Heus HA, Pardi A. 1991. Structural features that give rise to the unusual stability of RNA hairpins containing GNRA loops. *Science* **253**: 191–194.
- Heus HA, Formenoy LJ, Van Knippenberg PH. 1990. Conformational and thermodynamic effects of naturally occurring base methylations in a ribosomal RNA hairpin of *Bacillus stearothermophilus*. *Eur J Biochem* **188**: 275–281.
- Jucker FM, Heus HA, Yip PF, Moors EH, Pardi A. 1996. A network of heterogeneous hydrogen bonds in GNRA tetraloops. *J Mol Biol* **264**: 968–980.
- Kurata S, Weixlbaumer A, Ohtsuki T, Shimazaki T, Wada T, Kirino Y, Takai K, Watanabe K, Ramakrishnan V, Suzuki T. 2008. Modified uridines with C5-methylene substituents at the first position of the tRNA anticodon stabilize U-G wobble pairing during decoding. *J Biol Chem* **283**: 18801–18811.
- McCloskey JA, Rozenski J. 2005. The Small Subunit rRNA Modification Database. *Nucleic Acids Res* **33**: D135–D138.
- Moazed D, Van Stolk BJ, Douthwaite S, Noller HF. 1986. Interconversion of active and inactive 30S ribosomal subunits is accompanied by a conformational change in the decoding region of 16S rRNA. *J Mol Biol* **191**: 483–493.
- O'Connor M, Thomas CL, Zimmermann RA, Dahlberg AE. 1997. Decoding fidelity at the ribosomal A and P sites: Influence of mutations in three different regions of the decoding domain in 16S rRNA. *Nucleic Acids Res* **25**: 1185–1193.
- Ogle JM, Brodersen DE, Clemons WM Jr, Tarry MJ, Carter AP, Ramakrishnan V. 2001. Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science* **292**: 897–902.
- Ogle JM, Murphy FV, Tarry MJ, Ramakrishnan V. 2002. Selection of tRNA by the ribosome requires a transition from an open to a closed form. *Cell* **111**: 721–732.
- Otwinowski Z, Minor W. 1997. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* **276**: 307–326.
- Poldermans B, Goosen N, Van Knippenberg PH. 1979. Studies on the function of two adjacent N⁶,N⁶-dimethyladenosines near the 3' end of 16S ribosomal RNA of *Escherichia coli*. I. The effect of kasugamycin on initiation of protein synthesis. *J Biol Chem* **254**: 9085–9089.
- Rife JP, Moore PB. 1998. The structure of a methylated tetraloop in 16S ribosomal RNA. *Structure* **6**: 747–756.
- Rife JP, Cheng CS, Moore PB, Strobel SA. 1998. N²-methylguanosine is iso-energetic with guanosine in RNA duplexes and GNRA tetraloops. *Nucleic Acids Res* **26**: 3640–3644.
- Schluzen F, Takemoto C, Wilson DN, Kaminishi T, Harms JM, Hanawa-Suetsugu K, Szaflarski W, Kawazoe M, Shirouzu M, Nierhaus KH, et al. 2006. The antibiotic kasugamycin mimics mRNA nucleotides to destabilize tRNA binding and inhibit canonical translation initiation. *Nat Struct Mol Biol* **13**: 871–878.
- Schuwirth BS, Day JM, Hau CW, Janssen GR, Dahlberg AE, Cate JH, Vila-Sanjurjo A. 2006. Structural analysis of kasugamycin inhibition of translation. *Nat Struct Mol Biol* **13**: 879–886.
- Thammana P, Cantor CR. 1978. Studies on ribosome structure and interactions near the m⁶Am⁶2A sequence. *Nucleic Acids Res* **5**: 805–823.

- Thammana P, Held WA. 1974. Methylation of 16S rRNA during ribosome assembly in vitro. *Nature* **251**: 682–686.
- van Buul CP, Visser W, van Knippenberg PH. 1984. Increased translational fidelity caused by the antibiotic kasugamycin and ribosomal ambiguity in mutants harbouring the *ksgA* gene. *FEBS Lett* **177**: 119–124.
- Van Charldorp R, Heus HA, Van Knippenberg PH, Joordens J, De Bruin SH, Hilbers CW. 1981. Destabilization of secondary structure in 16S ribosomal RNA by dimethylation of two adjacent adenosines. *Nucleic Acids Res* **9**: 4413–4422.
- Van Knippenberg PH, Van Kimmenade JM, Heus HA. 1984. Phylogeny of the conserved 3' terminal structure of the RNA of small ribosomal subunits. *Nucleic Acids Res* **12**: 2595–2604.
- Vila-Sanjurjo A, Dahlberg AE. 2001. Mutational analysis of the conserved bases C1402 and A1500 in the center of the decoding domain of *Escherichia coli* 16S rRNA reveals an important tertiary interaction. *J Mol Biol* **308**: 457–463.
- Vila-Sanjurjo A, Squires CL, Dahlberg AE. 1999. Isolation of kasugamycin resistant mutants in the 16S ribosomal RNA of *Escherichia coli*. *J Mol Biol* **293**: 1–8.
- Wimberly BT, Brodersen DE, Clemons WM Jr, Morgan-Warren RJ, Carter AP, Vornrhein C, Hartsch T, Ramakrishnan V. 2000. Structure of the 30S ribosomal subunit. *Nature* **407**: 327–339.
- Woese CR, Winker S, Gutell RR. 1990. Architecture of ribosomal RNA: Constraints on the sequence of “tetra-loops.” *Proc Natl Acad Sci* **87**: 8467–8471.
- Xu Z, O'Farrell HC, Rife JP, Culver GM. 2008. A conserved rRNA methyltransferase regulates ribosome biogenesis. *Nat Struct Mol Biol* **15**: 534–536.
- Zamir A, Miskin R, Elson D. 1969. Interconversion between inactive and active forms of ribosomal subunits. *FEBS Lett* **3**: 85–88.