The 3D rRNA modification maps database: with interactive tools for ribosome analysis

Dorota Piekna-Przybylska, Wayne A. Decatur and Maurille J. Fournier*

Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA 01003, USA

ABSTRACT

The 3D rRNA modification maps database is the first general resource of information about the locations of modified nucleotides within the 3D structure of the full ribosome, with mRNA and tRNAs in the A-, P- and E-sites. The database supports analyses for several model organisms, including higher eukaryotes, and enables users to construct 3D maps for other organisms. Data are provided for human and plant (*Arabidopsis*) ribosomes, and for other representative organisms from eubacteria, archaea and eukarya. Additionally, the database integrates information about positions of modifications within rRNA sequences and secondary structures, as well as links to other databases and resources about modifications and their biosynthesis. Displaying positions of modified nucleotides is fully manageable. Views of each modified nucleotide are controlled by individual buttons and buttons also control the visibility of different ribosomal molecular components. A section called 'Paint Your Own' enables the user to create a 3D modification map for rRNA from any organism where sites of modification are known. This section also provides capabilities for visualizing nucleotides of interest in rRNA or tRNA, as well as particular amino acids in ribosomal proteins. The database can be accessed at http://people.biochem.umass.edu/fournierlab/3dmodmap/

INTRODUCTION

Post-transcriptionally altered nucleotides are common in ribosomal RNA (rRNA) in all organisms; however, prokaryotes and eukaryotes differ significantly in the numbers and types of these modifications (1–6). For example, the rRNA of *Escherichia coli* contains a total of 35 modifications. Most (69%) are methylated, with CH_3 groups added to heterocyclic bases (mN; 60%), and in a few cases to the ribose moiety (Nm; 9%). Another subset is comprised of uridines converted to pseudouridines (Ψ; 31%) (2,7). In eukaryotes the number of modifications in rRNA is significantly higher, with over 100 in yeast and more than 200 in vertebrates (3,6). The most abundant modifications in eukaryotic rRNAs are pseudouridines and ribose methylations, which typically account for ∼95% of the alterations. Less numerous are nucleotides with a base methylation(s).

Many protein and RNA factors that participate in rRNA modification have been defined and many remain to be identified (6,8–10). Thus, far eubacteria appear to use protein-only enzymes to catalyse the various modifications. However, while eukaryotes and archaea also have such enzymes, most $2'$-O-methylations and pseudouridines are created (respectively) by C/D and H/ACA families of small ribonucleoprotein complexes. The RNP complexes in each family consist of one unique, site-specific guide RNA and a small set of family-specific proteins (3 or 4). The guide RNA component identifies the nucleotide to be modified based on complementarity between the guide element(s) in the small RNA and the rRNA substrate (11–14).

The discovery that *Saccharomyces cerevisiae* C/D and H/ACA small nucleolar RNAs (snoRNAs) guide Nm and Ψ formation in rRNA (15–17) opened the way for identifying nearly all of the modifying snoRNPs and sites of Nm and Ψ in the first eukaryotic organism. Knowledge about the components of the yeast C/D and H/ACA snoRNPs spawned novel computational and experimental approaches for discovering guide snoRNAs and archaeal homologs (sRNAs) in many organisms (18–30). Exploiting the complementarities between the guide sequence(s) and rRNA by computational approaches has identified both novel guide RNAs and sites of modification (including non-rRNA substrates). Indeed, databases for plant snoRNAs and human snoRNAs (and the functionally related scaRNAs) contain large amounts of data about predicted and confirmed snoRNAs and potential targets (9,10). Knowledge about snoRNAs from other species is also developing quickly (31–37). Thus, often the number and positions of many rRNA modifications can be predicted for several organisms before they have been established experimentally.

*To whom correspondence should be addressed. Tel: +1 413 545 0566; Fax: +1 413 545 3291; E-mail: 4nier@biochem.umass.edu

Little is yet known about the significance of the modifications in ribosomal RNA, although targeted depletion studies have shown impaired behavior in a few cases (e.g. (38,39)). Strikingly, modeling of modification data of *E. coli*, archaea and *S. cerevisiae* into ribosome crystal structures revealed that many are concentrated in functionally important regions, for example, the peptidyl transferase center (PTC) in the large subunit (LSU), the decoding center in the small subunit (SSU) and on interacting surfaces of the two subunits (40–42). Although atomic-resolution structures of ribosomes from higher eukaryotes are not yet available, 3D modification maps can be developed from such organisms, by modeling modification data onto high-resolution structures that do exist. The merit of this approach is supported by the very highly conserved structure of the ribosome and its functions (43–46). While we eagerly await the availability of 3D maps that have been experimentally derived, development of deduced maps of the type described here will allow structure and function studies of modification effects to proceed at a high level.

DATABASE CONTENT

The 3D rRNA modification maps database builds on a database established by us for *S. cerevisiae* snoRNAs and rRNA modifications, which pioneered the deduced mapping approach used in the present study. The new database features nucleotide modification data for several major model organisms and new tools for creating and analysing fully manageable 3D modification maps for rRNA from these and other sources. The tools provide full models of the ribosome at 2.8 and 5.5 Å resolutions, with both subunits, and mRNA and tRNAs in the A-, P- and E-sites. A specialized interface exists for controlling the visibility of the modified nucleotides and the different molecular components of the ribosome. The database supports analyses for several representative organisms from the three kingdoms, i.e. the eubacteria (*E. coli*, *Thermus thermophilus*), archaea (*Haloarcula marismortui*) and eukarya (human, *Arabidopsis*, *S. cerevisiae*). A section called 'Paint Your Own' enables users to construct 3D modification maps for other organisms of interest. The new database also allows visualization of nucleotides of interest in rRNA or tRNA, and particular amino acids in ribosomal proteins.

Ribosomal modifications of representative species are also displayed within the rRNA sequences and on rRNA secondary (2D) structure maps. Details about guide RNAs, core RNP proteins and proteins known or predicted to mediate modification are also provided. The database is heavily linked to many other web resources, including yeast, plant and human snoRNA databases (6,9,10), the RNA modification database (2), Modomics—a database of RNA modification pathways (8), SGD—the *Saccharomyces* genome database (47) and EcoCyc—a comprehensive genome database for *E. coli* (48).

DATABASE ORGANIZATION, INTERFACE AND ACCESS

The 3D rRNA modification maps database is accessible on the World Wide Web at http://people.biochem.umass.edu/fournierlab/3dmodmap/. It is designed to facilitate 3D visualization of rRNA sites modified in many different organisms, and to provide quick access to detailed information available about these modifications. For the sake of consistency with human and plant analyses, we have adopted rRNA sequences and nucleotide numbering systems used in the human and plant snoRNA databases (http://www-snorna.biotoul.fr/, http://bioinf.scri.sari.ac.uk/cgi-bin/plant_snorna/home).

Displaying modifications

The centerpiece of the database is the section called 'Mod sites in 3D'; however, three other sections 'Mods in Seq.', 'Mods in 2D Str.' and 'Equiv. Sites' are essential supplements since they determine the position of the modified nucleotides in the 3D structures. A short description of each section follows:

(i) 'Mods in Seq.' provides sequences of ribosomal RNAs with the sites of modification highlighted. If a known or predicted protein or snoRNA is assigned a role in the formation of the modification, this information is also indicated and the user is referred to other pertinent web resources. For example, C967 in the 16S rRNA of *E. coli* is methylated to m^5C by the methyltransferase RsmB. The C967 link leads to entries for the RsmB protein in two other databases: Modomics and EcoCyc, as well as literature citations in PubMed (Figure 1). The same approach is used for modifications guided by human and plant snoRNAs. In these cases, links direct the user to appropriate entries for the corresponding snoRNAs in the human snoRNA database (snoRNA-LBME-db (9)) and plant snoRNA database (10). For *S. cerevisiae*, the user has direct access to appropriate pages in a separate database for yeast snoRNAs (6), which provides further details about the respective guide snoRNAs.

(ii) 'Mods in 2D Str.' Here, modifications are displayed for selected organisms on rRNA secondary structure diagrams. As noted above, the 2D structures annotated with modification sites for human and plant rRNAs were prepared using the same rRNAs that appear in the human and plant snoRNA databases.

(iii) 'Mod sites in 3D' provides the user with tools for displaying fully manageable 3D ribosomal modification maps. Because the interface is based on Jmol (49), only a JAVA-capable browser is needed. Pages designed for highlighting the positions of modifications in three dimensions contain a window with a loaded ribosome structure on the right and controls on the left. We feature two models of the *T. thermophilus* 70S ribosome resolved at 2.8 and 5.5 Å resolution; both contain tRNAs at the A-, P- and E-sites, and a fragment of mRNA (45,50).
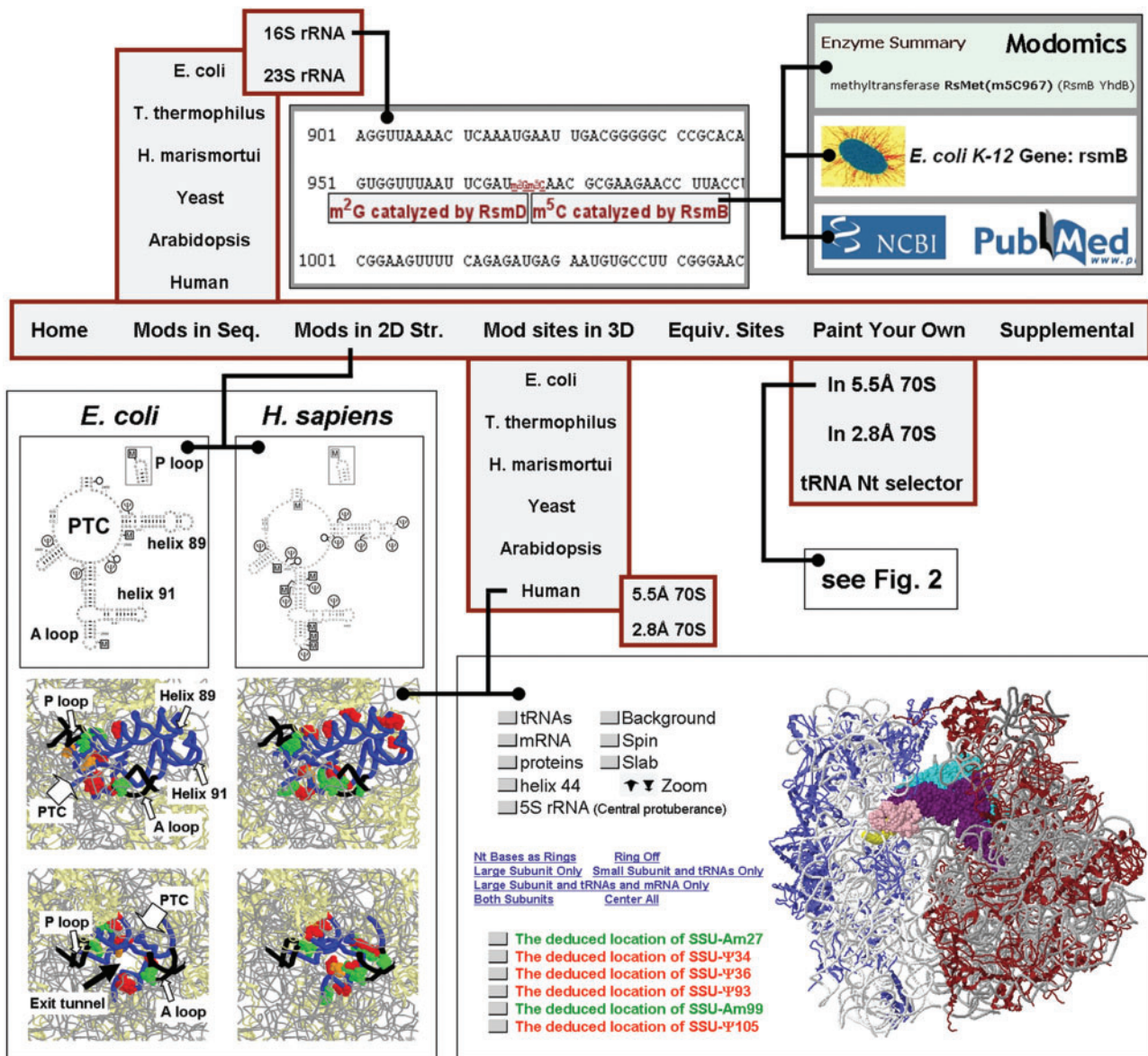
Figure 1. The positions of the modified nucleotides in the rRNA of several well-studied organisms can be visualized in linear, secondary structure and 3D structure formats. The menu bar (top) shows the sections of the database, and portions of the associated links are illustrated in exploded views. The examples shown are for modifications concentrated around the A site and PTC regions of *E. coli* and human (middle left; upper, view of the A site and PTC, lower, view of the PTC toward the exit tunnel). The 3D arrangements of the modified nucleotides in *E. coli* and human rRNAs are displayed using bioinformatics tools (middle right) available in a section named 'Mod sites in 3D'. An interactive interface is provided for controlling the views of the ribosome model (both subunits), and the A-, P- and E-site tRNAs, and mRNA) and for selecting modifications, as follows: 'pseudouridines' (red), '2′-*O*-methylations' (green) and 'base methylations' (orange). The web service is heavily linked to material in other databases and web resources, for example, snoRNA databases for human, yeast and plants, Modomics (a database of RNA modification pathways), EcoCyc (a comprehensive genome database for *E. coli*) and PubMed.

The 2.8 Å structure provides greater detail; however, only a fragment of the anticodon stem–loop of the tRNA bound at the A site is visible due to disorder.

A specialized interface on the left controls the interactive views of the ribosome model. A panel of buttons makes convenient manipulations that control, for example, the degree of zoom, whether the model constantly spins, and the visibility of different ribosomal components such as the tRNAs, ribosomal proteins or a relevant subunit. Buttons for highlighting 5S rRNA in the central protuberance of the LSU and helix 44 on the intersubunit surface of the SSU, facilitate viewer orientation of the ribosome structure. Also featured on the left is a series of toggle buttons corresponding to each individual modified nucleotide, enabling highlighting of desired positions only, for example, those in the PTC (Figure 1). The buttons are numbered by position in the rRNA of the particular organism. Thus, the user need only have knowledge of the rRNA sequence of a particular organism, in order to

display the deduced positions of modified nucleotides in 3D. Tables provided in the next section list deduced positions in *E. coli* rRNAs for modifications of the representative organisms featured. In a few cases, modification positions cannot yet be displayed because of disorder in the relevant regions of the ribosome or lack of corresponding domains in prokaryotic rRNA. These positions are also identified.

(iv) 'Equiv. Sites'. All mapped modifications in the representative organisms are listed in this section. In addition, rRNA positions proposed to be modified on the basis of a guide element found in a predicted snoRNA are also noted. Importantly, for each of these modifications we list equivalent and near-equivalent nucleotide in *E. coli* and *H. marismortui* rRNAs, as deduced by us. We also indicate whether modifications are present in corresponding positions in these organisms, as well as in yeast and human rRNAs.

Accuracy and completeness of the database

Putative snoRNAs with sequences complementary to rRNA may not actually be involved in guiding modification. This situation is especially relevant to plant and human snoRNAs where candidates are quite numerous. Since the modification status of each nucleotide in plant and human ribosomal RNAs is not yet known conclusively we have only indicated in the rRNA sequences and 2D structure diagrams modifications that are documented and those that are strongly predicted but not confirmed. The latter decision was based on two factors: assignment of a potential guide snoRNA to a position in rRNA, and the existence of the same modification in the same position in the rRNA of another eukaryote(s).

Predicted modifications are not marked if only a potential guide snoRNA gene has been assigned. For example, 14 putative guide snoRNAs have been identified in humans for which no modification data yet exist. However, modifications have been confirmed in *S. cerevisiae* for three of the predicted human sites and on this basis we have highlighted the corresponding positions in human rRNA. Such modifications are distinguished in the 2D rRNA diagrams by gray highlighting in contrast to verified modifications, which are indicated in black. Gray highlighting is also used to indicate modifications that are not conclusively mapped to the resolution of a specific nucleotide. This last category includes 6 Ψ and 4 Nm modifications in human rRNA. In the literature these modifications were assigned to two different nucleotides, but we have assigned them to specific positions in the RNA sequences and 2D diagrams on the basis of the predicted pairings with the guide elements of the assigned snoRNAs proposed; in four cases the assignment is strengthened by the presence of the same modification in the same corresponding position in yeast. These details are noted in footnotes for the tables in the 'Equiv. Sites' section. The pages displaying the 3D modification maps in the 'Mod sites in 3D' section have toggle controls for highlighting the complete inventory of

modifications that are: Confirmed, Not Confirmed—but supported by modification data for another organism(s) and Proposed.

PAINT YOUR OWN

While our database is designed for visualizing modified ribosomal nucleotides of human, *Arabidopsis*, *S. cerevisiae*, *H. marismortui*, *E. coli* and *T. thermophilus*, it is not limited to 3D modification maps for these organisms. The user can also 'paint' or specifically highlight positions of modifications (or other sites) in rRNAs of other species as well, as permitted by the sequence and modification data available. A section 'Paint Your Own' shows users how to create their own 3D modification map using the Jmol console (Figure 2). We lead the user to pages with codes of ribosomal molecules for both 70S models, and describe how to display desired portions of the ribosome in various formats using the appropriate commands.

One example of a potential application is construction of a 3D map as follows for a specific region of rRNA in the archaeon *Pyrococcus abyssi*. In this species, some 46 genes encoding putative guide small RNAs (sRNAs) have been identified and confirmed to be expressed; these sRNAs contain box C- and D-like sequences and guide elements predicted to target methylation to rRNA (51). For another archaeon, *Pyrococcus furiosus*, two sRNAs were shown to guide methylation *in vivo* to the expected sites in a eukaryotic rRNA (52). Three of the predicted guide sRNAs from *P. abyssi* (sR1, sR4 and sR29) are proposed to guide five methylations within the GTPase center of the LSU (G1200, C1216, C1233, C1238 and C1240). To display the positions of these predicted methylations in a 3D model of the ribosome, equivalent nucleotides should first be identified on a secondary structure rRNA map of *E. coli*. This numbering system is used for the *T. thermophilus* rRNA in the 70S crystal models and the *E. coli* equivalents for the five *P. abyssi* nucleotides in 23S rRNA are readily deduced (G1059, C1075, C1092, U1097 and C1100). These positions are not visible in the 2.8 Å ribosome model due to disorder in that part of the structure; however, they can be displayed in the 70S model at 5.5 Å. Only a few commands are needed in an easily accessed Jmol console to visualize the positions of interest (Figure 2).

From a tutorial delineating the 3D mapping process in greater detail, we direct users to specific pages in the Protein Data Bank (PDB; www.pdb.org) for information about designations of ribosomal molecules (proteins, rRNAs, tRNAs, mRNA) and disordered portions in both ribosome models employed in the database. With these details and our guide, a user may visualize any nucleotides of interest in rRNA, as well as particular amino acids in ribosomal proteins being considered. Examples of applications where these capabilities could be valuable include designing mutagenesis experiments and evaluating cross-linking and genetic suppressor data. A separate subsection called 'tRNA Nt Selector' facilitates 3D visualization of tRNA nucleotides, for example modified nucleotides within ribosome-bound tRNAs.
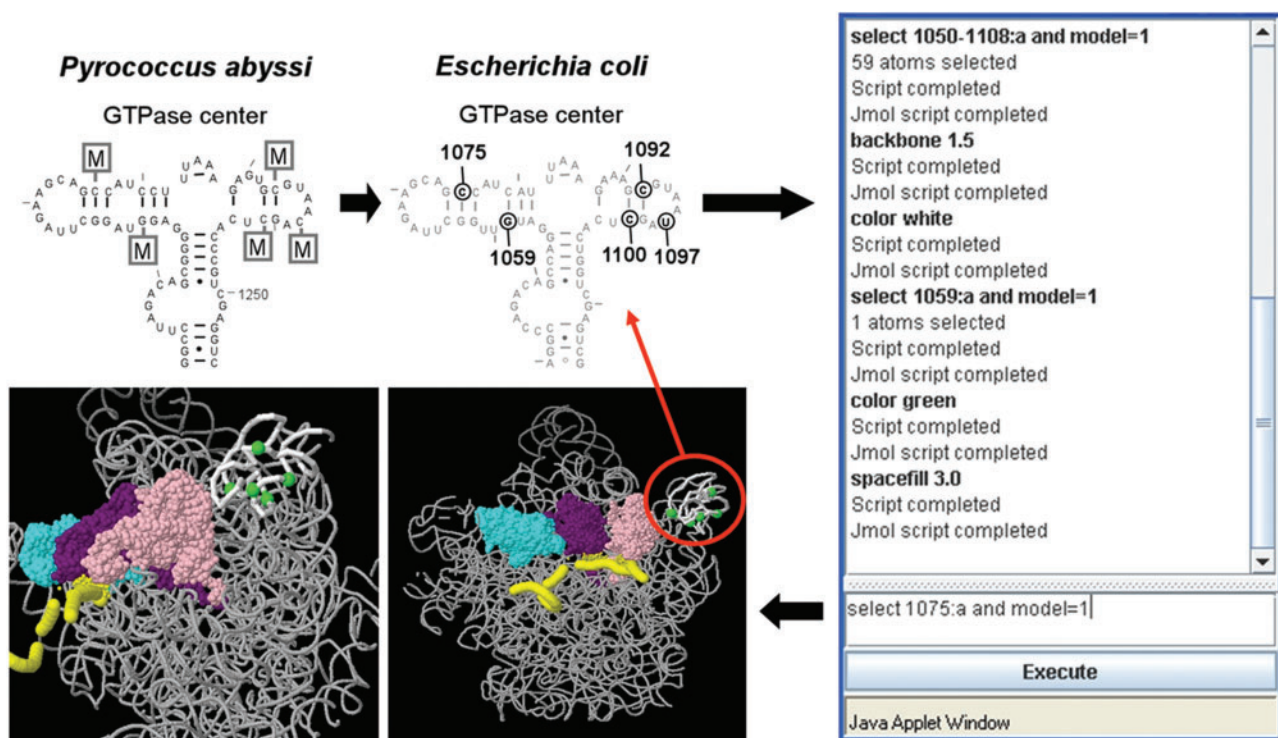
Figure 2. Paint Your Own. Users may create 3D modification maps for rRNA from any organism. The 'Paint Your Own' section with an accompanying tutorial enables selection and highlighting of any site or segment within an rRNA, r-protein and tRNA. The example shown is for a deduced 3D arrangement of predicted 2′-O-methylations concentrated within the GTPase center of *P. abyssi*, using the ribosome model at 5.5 Å. Stepwise, visualization was achieved by: (i) determining the equivalent numbers of the rRNA nucleotides from the secondary structure of *E. coli* 23S rRNA (53) and, (ii) selecting and highlighting the GTPase center and the positions of the modified nucleotides, using a few commands provided in the Jmol console window. The rRNA fragment of the GTPase center is shown as a thick white backbone, and modified nucleotides are displayed as green dots. The tRNAs are placed in the A- (pink), P- (purple) and E- (cyan) sites of the LSU. A fragment of mRNA within the small ribosomal subunit (hidden) is displayed in yellow. Proteins of the LSU are also hidden.

ACKNOWLEDGEMENTS

REFERENCES

1. Lapeyre,B. (2005) Conserved ribosomal RNA modification and their putative roles in ribosome biogenesis and translation. In Grosjean,H. (ed.), *Topics in Current Genetics: Fine-Tuning of RNA Functions by Modification and Editing*, Vol. 12, Springer-Verlag, Berlin/Heidelberg/New York, pp. 263–284.
2. Rozenski,J., Crain,P.F. and McCloskey,J.A. (1999) The RNA modification database: 1999 update. *Nucleic Acids Res.*, **27**, 196–197.
3. Maden,B.E. (1990) The numerous modified nucleotides in eukaryotic ribosomal RNA. *Prog. Nucleic Acid Res. Mol. Biol.*, **39**, 241–303.
4. Ofengand,J. and Bakin,A. (1997) Mapping to nucleotide resolution of pseudouridine residues in large subunit ribosomal RNAs from representative eukaryotes, prokaryotes, archaebacteria, mitochondria and chloroplasts. *J. Mol. Biol.*, **266**, 246–268.
5. McCloskey,J.A. and Rozenski,J. (2005) The small subunit rRNA modification database. *Nucleic Acids Res.*, **33**, D135–D138.
6. Piekna-Przybylska,D., Decatur,W.A. and Fournier,M.J. (2007) New bioinformatic tools for analysis of nucleotide modifications in eukaryotic rRNA. *RNA*, **13**, 305–312.
7. Del Campo,M., Kaya,Y. and Ofengand,J. (2001) Identification and site of action of the remaining four putative pseudouridine synthases in *Escherichia coli*. *RNA*, **7**, 1603–1615.
8. Dunin-Horkawicz,S., Czerwoniec,A., Gajda,M.J., Feder,M., Grosjean,H. and Bujnicki,J.M. (2006) MODOMICS: a database of RNA modification pathways. *Nucleic Acids Res.*, **34**, D145–D149.
9. Lestrade,L. and Weber,M.J. (2006) snoRNA-LBME-db, a comprehensive database of human H/ACA and C/D box snoRNAs. *Nucleic Acids Res.*, **34**, D158–D162.
10. Brown,J.W., Echeverria,M., Qu,L.H., Lowe,T.M., Bachellerie,J.P., Huttenhofer,A., Kastenmayer,J.P., Green,P.J., Shaw,P. *et al*. (2003) Plant snoRNA database. *Nucleic Acids Res.*, **31**, 432–435.
11. Bertrand,E. and Fournier,M.J. (2004) The snoRNPs and related machines: ancient devices that mediate maturation of rRNA and other RNAs. In Olson,M.O.J. (ed.), *The Nucleolus*. Eurekah.com and Kluwer Academic/Plenum Publishers, Georgetown, TX; New York, pp. 223–257.
12. Kiss,T. (2004) Biogenesis of small nuclear RNPs. *J. Cell Sci.*, **117**, 5949–5951.
13. Meier,U.T. (2005) The many facets of H/ACA ribonucleoproteins. *Chromosoma*, **114**, 1–14.
14. Yu,Y.-T., Terns,R.M. and Terns,M.P. (2005) Mechanisms and functions of RNA-guided RNA modification. In Grosjean,H. (ed.), *Topics in Current Genetics: Fine-Tuning of RNA Functions by Modification and Editing*, Vol. 12, Springer-Verlag, Berlin/Heidelberg/New York, pp. 223–262.
15. Kiss-Laszlo,Z., Henry,Y., Bachellerie,J.P., Caizergues-Ferrer,M. and Kiss,T. (1996) Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell*, **85**, 1077–1088.
16. Ni,J., Tien,A.L. and Fournier,M.J. (1997) Small nucleolar RNAs direct site-specific synthesis of pseudouridine in ribosomal RNA. *Cell*, **89**, 565–573.

17. Ganot,P., Bortolin,M.L. and Kiss,T. (1997) Site-specific pseudouridine formation in preribosomal RNA is guided by small nucleolar RNAs. *Cell*, **89**, 799–809.
18. Olivas,W.M., Muhlrad,D. and Parker,R. (1997) Analysis of the yeast genome: identification of new non-coding and small ORF-containing RNAs. *Nucleic Acids Res.*, **25**, 4619–4625.
19. Lowe,T.M. and Eddy,S.R. (1999) A computational screen for methylation guide snoRNAs in yeast. *Science*, **283**, 1168–1171.
20. Edvardsson,S., Gardner,P.P., Poole,A.M., Hendy,M.D., Penny,D. and Moulton,V. (2003) A search for H/ACA snoRNAs in yeast using MFE secondary structure prediction. *Bioinformatics*, **19**, 865–873.
21. McCutcheon,J.P. and Eddy,S.R. (2003) Computational identification of non-coding RNAs in *Saccharomyces cerevisiae* by comparative genomics. *Nucleic Acids Res.*, **31**, 4119–4128.
22. Schattner,P., Decatur,W.A., Davis,C.A., Ares,M.Jr, Fournier,M.J. and Lowe,T.M. (2004) Genome-wide searching for pseudouridyla-tion guide snoRNAs: analysis of the *Saccharomyces cerevisiae* genome. *Nucleic Acids Res.*, **32**, 4281–4296.
23. Schattner,P., Brooks,A.N. and Lowe,T.M. (2005) The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. *Nucleic Acids Res.*, **33**, W686–W689.
24. Gu,A.D., Zhou,H., Yu,C.H. and Qu,L.H. (2005) A novel experimental approach for systematic identification of box H/ACA snoRNAs from eukaryotes. *Nucleic Acids Res.*, **33**, e194.
25. Torchet,C., Badis,G., Devaux,F., Costanzo,G., Werner,M. and Jacquier,A. (2005) The complete set of H/ACA snoRNAs that guide rRNA pseudouridylations in *Saccharomyces cerevisiae*. *RNA*, **11**, 928–938.
26. Ghazal,G., Ge,D., Gervais-Bird,J., Gagnon,J. and Abou Elela,S. (2005) Genome-wide prediction and analysis of yeast RNase III-dependent snoRNA processing signals. *Mol. Cell. Biol.*, **25**, 2981–2994.
27. Schattner,P., Barberan-Soler,S. and Lowe,T.M. (2006) A computational screen for mammalian pseudouridylation guide H/ACA RNAs. *RNA*, **12**, 15–25.
28. Yang,J.H., Zhang,X.C., Huang,Z.P., Zhou,H., Huang,M.B., Zhang,S., Chen,Y.Q. and Qu,L.H. (2006) snoSeeker: an advanced computational package for screening of guide and orphan snoRNA genes in the human genome. *Nucleic Acids Res.*, **34**, 5112–5123.
29. Davis,C.A. and Ares,M.Jr. (2006) Accumulation of unstable promoter-associated transcripts upon loss of the nuclear exosome subunit Rrp6p in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **103**, 3262–3267.
30. Houalla,R., Devaux,F., Fatica,A., Kufel,J., Barrass,D., Torchet,C. and Tollervey,D. (2006) Microarray detection of novel nuclear RNA substrates for the exosome. *Yeast*, **23**, 439–454.
31. Huttenhofer,A., Kiefmann,M., Meier-Ewert,S., O'Brien,J., Lehrach,H., Bachellerie,J.P. and Brosius,J. (2001) RNomics: an experimental approach that identifies 201 candidates for novel, small, non-messenger RNAs in mouse. *EMBO J.*, **20**, 2943–2953.
32. Marker,C., Zemann,A., Terhorst,T., Kiefmann,M., Kastenmayer,J.P., Green,P., Bachellerie,J.P., Brosius,J. and Huttenhofer,A. (2002) Experimental RNomics: identification of 140 candidates for small non-messenger RNAs in the plant *Arabidopsis thaliana*. *Curr. Biol.*, **12**, 2002–2013.
33. Yuan,G., Klambt,C., Bachellerie,J.P., Brosius,J. and Huttenhofer,A. (2003) RNomics in *Drosophila melanogaster*: identification of 66 candidates for novel non-messenger RNAs. *Nucleic Acids Res.*, **31**, 2495–2507.
34. Huang,Z.P., Zhou,H., He,H.L., Chen,C.L., Liang,D. and Qu,L.H. (2005) Genome-wide analyses of two families of snoRNA genes from *Drosophila melanogaster*, demonstrating the extensive utilization of introns for coding of snoRNAs. *RNA*, **11**, 1303–1316.
35. Liang,X.H., Uliel,S., Hury,A., Barth,S., Doniger,T., Unger,R. and Michaeli,S. (2005) A genome-wide analysis of C/D and H/ACA-like small nucleolar RNAs in *Trypanosoma brucei* reveals a trypanosome-specific pattern of rRNA modification. *RNA*, **11**, 619–645.
36. Liang,X.H., Hury,A., Hoze,E., Uliel,S., Myslyuk,I., Apatoff,A., Unger,R. and Michaeli,S. (2007) Genome-wide analysis of C/D and H/ACA-like small nucleolar RNAs in *Leishmania* major indicates conservation among trypanosomatids in the repertoire and in their rRNA targets. *Eukaryot. Cell*, **6**, 361–377.
37. Xie,J., Zhang,M., Zhou,T., Hua,X., Tang,L. and Wu,W. (2007) Sno/scaRNAbase: a curated database for small nucleolar RNAs and cajal body-specific RNAs. *Nucleic Acids Res.*, **35**, D183–D187.
38. King,T.H., Liu,B., McCully,R.R. and Fournier,M.J. (2003) Ribosome structure and activity are altered in cells lacking snoRNPs that form pseudouridines in the peptidyl transferase center. *Mol. Cell*, **11**, 425–435.
39. Baxter-Roshek,J.L., Petrov,A.N. and Dinman,J.D. (2007) Optimization of ribosome structure and function by rRNA base modification. *PLoS ONE*, **2**, e174.
40. Ofengand,J. (2002) Ribosomal RNA pseudouridines and pseudouridine synthases. *FEBS Lett.*, **514**, 17–25.
41. Decatur,W.A. and Fournier,M.J. (2002) rRNA modifications and ribosome function. *Trends Biochem. Sci.*, **27**, 344–351.
42. Omer,A.D., Ziesche,S., Decatur,W.A., Fournier,M.J. and Dennis,P.P. (2003) RNA-modifying machines in archaea. *Mol. Microbiol.*, **48**, 617–629.
43. Spahn,C.M., Beckmann,R., Eswar,N., Penczek,P.A., Sali,A., Blobel,G. and Frank,J. (2001) Structure of the 80S ribosome from *Saccharomyces cerevisiae*—tRNA-ribosome and subunit–subunit interactions. *Cell*, **107**, 373–386.
44. Spahn,C.M., Gomez-Lorenzo,M.G., Grassucci,R.A., Jorgensen,R., Andersen,G.R., Beckmann,R., Penczek,P.A., Ballesta,J.P. and Frank,J. (2004) Domain movements of elongation factor eEF2 and the eukaryotic 80S ribosome facilitate tRNA translocation. *EMBO J.*, **23**, 1008–1019.
45. Yusupov,M.M., Yusupova,G.Z., Baucom,A., Lieberman,K., Earnest,T.N., Cate,J.H. and Noller,H.F. (2001) Crystal structure of the ribosome at 5.5 Å resolution. *Science*, **292**, 883–896.
46. Ban,N., Nissen,P., Hansen,J., Moore,P.B. and Steitz,T.A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science*, **289**, 905–920.
47. Dwight,S.S., Balakrishnan,R., Christie,K.R., Costanzo,M.C., Dolinski,K., Engel,S.R., Feierbach,B., Fisk,D.G., Hirschman,J. *et al.* (2004) *Saccharomyces* genome database: underlying principles and organisation. *Brief. Bioinform.*, **5**, 9–22.
48. Keseler,I.M., Collado-Vides,J., Gama-Castro,S., Ingraham,J., Paley,S., Paulsen,I.T., Peralta-Gil,M. and Karp,P.D. (2005) EcoCyc: a comprehensive database resource for *Escherichia coli*. *Nucleic Acids Res.*, **33**, D334–D337.
49. Herráez,A. (2006) Biomolecules in the computer. Jmol to the rescue. *Biochem. Mol. Biol. Educ.*, **34**, 255–261.
50. Selmer,M., Dunham,C.M., Murphy,F.V.T., Weixlbaumer,A., Petry,S., Kelley,A.C., Weir,J.R. and Ramakrishnan,V. (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. *Science*, **313**, 1935–1942.
51. Gaspin,C., Cavaille,J., Erauso,G. and Bachellerie,J.P. (2000) Archaeal homologs of eukaryotic methylation guide small nucleolar RNAs: lessons from the *Pyrococcus* genomes. *J. Mol. Biol.*, **297**, 895–906.
52. Speckmann,W.A., Li,Z.H., Lowe,T.M., Eddy,S.R., Terns,R.M. and Terns,M.P. (2002) Archaeal guide RNAs function in rRNA modification in the eukaryotic nucleus. *Curr. Biol.*, **12**, 199–203.
53. Cannone,J.J., Subramanian,S., Schnare,M.N., Collett,J.R., D'Souza,L.M., Du,Y., Feng,B., Lin,N., Madabusi,L.V. *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.