Nucleic Acids Research, 2001, Vol. 29, No. 1 171–172

tmRDB (tmRNA database)

Bjarne Knudsen, Jacek Wower[1], Christian Zwieb[2,*] and Jan Gorodkin

Department of Genetics and Ecology, The Institute of Biological Sciences, University of Aarhus, Building 540, Ny Munkegade, DK-8000 Aarhus C, Denmark, [1]Department of Animal and Dairy Sciences, Program in Cell and Molecular Biosciences, Auburn University, Auburn, AL 36849-5415, USA and [2]Department of Molecular Biology, The University of Texas Health Science Center at Tyler, 11937 US Highway 271, Tyler, TX 75708-3154, USA

Received September 20, 2000; Accepted September 27, 2000

ABSTRACT

The tmRNA database (tmRDB) is maintained at the University of Texas Health Science Center at Tyler, Texas, and accessible on the World Wide Web at the URL http://psyche.uthct.edu/dbs/tmRDB/tmRDB.html. Mirror sites are located at Auburn University, Auburn, Alabama (http://www.ag.auburn.edu/mirror/tmRDB/) and the Institute of Biological Sciences, Aarhus, Denmark (http://www.bioinf.au.dk/tmRDB/). The tmRDB provides information and citation links about tmRNA, a molecule that combines functions of tRNA and mRNA in *trans*-translation. tmRNA is likely to be present in all bacteria and has been found in algae chloroplasts, the cyanelle of *Cyanophora paradoxa* and the mitochondrion of the flagellate *Reclinomonas americana*. This release adds 26 new sequences and corresponding predicted tmRNA-encoded tag peptides for a total of 86 tmRNAs, ordered alphabetically and phylogenetically. Secondary structures and three-dimensional models in PDB format for representative molecules are being made available. tmRNA alignments prove individual base pairs and are generated manually assisted by computational tools. The alignments with their corresponding structural annotation can be obtained in various formats, including a new column format designed to improve and simplify computational usability of the data.

tmRDB TABLE OF CONTENTS

tmRNA FUNCTION

In *trans*-translation, a ribosome bound to the 3′-end of damaged mRNA moves to the resume codon and tag-peptide coding region of tmRNA, terminates at the tmRNA-encoded stop signal and releases a polypeptide which is digested by cytoplasmic and periplasmic proteases (1). Because of its tRNA-like properties, tmRNA can be charged with alanine, form a ternary complex with elongation factor Tu and enter the ribosomal A-site (for reviews, see 2–4). The binding of tmRNA to ribosomes is facilitated by protein SmpB (5) and ribosomal protein S1 (6).

tmRDB DESCRIPTION

tmRDB provides aligned, annotated and phylogenetically ordered sequences related to tmRNA structure and function. New and updated tmRNAs were obtained by using a representative subset of sequences as input for BLASTN to search GenBank (7). The search was automated by using a wrapper which performed BLASTN searches and GenBank sequence retrieval. Search and retrieval (from the wrapper) were performed directly on NCBI servers via the Internet. The BLAST hits within the wrapper were filtered for already known hits. For the remaining hits, sequences were extended in both directions, and a global alignment with the entire sequences was executed using the align0 program (8,9). Sequences were obtained also by searching annotations in GenBank, numerous genome projects available on the Web and the tmRNA website (10).

The 26 new tmRNAs (for a total of 86 sequences) were from the following species (ordered alphabetically): *Acidithiobacillus ferroxidans*, *Bacillus anthracis*, *Bordetella bronchiseptica* (sequence contains ambiguous residues), *Bradyrhizobium japonicum*, *Caulobacter crescentus*, *Chlamydia muridarum*, *Chlamydophila psittaci*, *Corynebacterium diphtheriae*, *Desulfovibrio vulgaris*, *Enterococcus faecium*, *Fusobacterium nucleatum*, *Haemophilus ducreyi*, *Methylobacterium extorquens* (partial sequence), *Neisseria meningitidis*, *Nitrosomonas europaea*, *Nostoc punctiforme*, *Pseudomonas putida*, *Reclinomonas americana* mitochondrion, *Rhodopseudomonas palustris*, *Rickettsia prowazekii*, *Sinorhizobium meliloti*, *Staphylococcus epidermidis*, *Streptococcus equi*, *Streptomyces coelicolor*, *Ureaplasma urealyticum* and *Xylella fastidiosa*. It is significant that tmRNAs and tmRNA-like molecules have now been identified in all bacterial groups (11), including the

*To whom correspondence should be addressed. Tel: +1 903 877 7689, Fax: +1 903 877 5731; Email: zwieb@uthct.edu

Alpha-Proteobacteria and the mitochondria of the freshwater heterotrophic flagellate *Reclinomonas americana* (12). Because of flexible gene arrangements and RNA processing events, it appears premature to conclude that tmRNA-like molecules are absent in Archaea and Eucarya (11).

Described criteria (13) were used to incorporate new sequences into the tmRNA alignment (14) and to derive secondary structures which include base pairs supported by comparative sequence analysis, thus avoiding procedures which maximize base pairing (10,15). Inclusion of new sequences into the previous alignment confirmed 17 helices (not every helix is present in every molecule), tRNA- and mRNA-like portions, and four pseudoknots (pk1 to pk4) (16). Local improvements in certain previously ambiguous regions were achieved.

The tmRDB shows the updated alignment in a variety of convenient formats. New to this update is the inclusion of a column format, which lists the sequences in separate entries. Each entry contains a header specifying properties related to the alignment. Computer programs can easily read the headers associated with various columns. These include the sequence, nucleotide positions in the alignment and positions of base pairs. A number of programs can assist the manual update of the alignments by checking, for example, base pairing consistency, phylogenetic support and possible extensions of base paired regions (manuscript in preparation).

The tmRDB also provides alignments of predicted tmRNA encoded tag peptides, numerous tmRNA secondary structure models and tentative three-dimensional models in PDB format generated interactively with the RNA modeling software ERNA-3D (17) and refined by energy minimization with VCMD (18). Work is in progress to include more details about tmRNA-associated proteins alanyl-tRNA synthetase, elongation factor Tu, SmpB and ribosomal protein S1.

ACCESS

The data are accessible freely for research purposes at the URL http://psyche.uthct.edu/dbs/tmRDB/tmRDB.html and mirror sites http://www.ag.auburn.edu/mirror/tmRDB/ and http://www.bioinf.au.dk/tmRDB/. Hard copies of tmRNA and tag-peptide alignments are available by Email request to the third author at zwieb@uthct.edu or through written contact. The first and last authors can be reached at the Email address bk@bioinf.au.dk and gorodkin@bioinf.au.dk, respectively. J. Wower can be contacted at jwower@acesag.auburn.edu. This article should be cited in research projects assisted by the use of tmRDB.

ACKNOWLEDGEMENTS

We thank Florian Müller for ERNA-3D and Ingolf Sommer for VCMD software. This work was supported by NIH grant GM-58267 to J.W. J.G. is supported by the Danish Technical Research Council.

REFERENCES

1. Keiler,K.C., Waller,P.R.H. and Sauer,R.T. (1996) Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA. *Science*, **271**, 990–993.
2. Muto,A., Ushida,C. and Himeno,H. (1998) A bacterial RNA that functions as both a tRNA and an mRNA. *Trends Biochem. Sci.*, **1**, 25–29.
3. Gottesman,S., Roche,E., Zhou,Y. and Sauer,R.T. (1998) The ClpXP and ClpAP proteases degrade proteins with carboxy-terminal peptide tails added by the SsrA-tagging system. *Genes Dev.*, **12**, 1338–1347.
4. Karzai,A.W., Roche,E.D. and Sauer,R.T. (2000) The SsrA-SmpB system for protein tagging, directed degradation and ribosome rescue. *Nature Struct. Biol.*, **7**, 449–455.
5. Karzai,A.W., Susskind,M.M. and Sauer,R.T. (1999) SmpB, a unique RNA-binding protein essential for the peptide-tagging activity of SsrA (tmRNA). *EMBO J.* **18**, 3793–3799.
6. Wower,J., Zwieb,C. and Wower,I.K. (2000) tmRNA: a case of structural and functional mimicry on and off the ribosome? In Garrett,R.A., Douthwaite,S.R., Liljas,A., Matheson,A.T., Moore,P.B. and Noller,H.F. (eds), *The Ribosome: Structure, Function, Antibiotics, and Cellular Interactions*. ASM Press, Washington, DC, pp. 397–405.
7. Benson,D.A., Karsch-Mizrachi,I., Lipman,D.J., Ostell,J., Rapp,B.A. and Wheller,D.L. (2000) GenBank. *Nucleic Acids Res.*, **28**, 15–18.
8. Myers,E.W. and Miller,W. (1988) Optimal alignments in linear space. *Comput. Appl. Biosci.*, **4**, 11–17.
9. Pearson,W.R. (1990) Rapid and sensitive sequence comparison with FASTP and FASTA. **183**, 63–98.
10. Williams,K.P. (2000) The tmRNA Website. *Nucleic Acids Res.*, **28**, 168.
11. Keiler,K.C., Shapiro,L. and Williams,K.P. (2000) tmRNAs that encode proteolysis-inducing tags are found in all known bacterial genomes: A two-piece tmRNA functions in Caulobacter. *Proc. Natl Acad. Sci. USA*, **97**, 7778–7783.
12. Lang,B.F., Seif,E., Gray,M.W., O'Kelly,C.J. and Burger G. (1999) A comparative genomics approach to the evolution of eukaryotes and their mitochondria. *J. Eukaryot. Microbiol.*, **46**, 320–326.
13. Larsen,N. and Zwieb,C. (1991) SRP-RNA sequence alignment and secondary structure. *Nucleic Acids Res.*, **19**, 209–215.
14. Zwieb,C. and Wower,J. (2000) tmRDB (tmRNA database). *Nucleic Acids Res.* **28**, 169–170.
15. Williams,K.P. and Bartel,D. (1996) Phylogenetic analysis of tmRNA secondary structure. *RNA*, **2**, 1306–1310.
16. Zwieb,C., Wower,I. and Wower,J. (1999) Comparative sequence analysis of tmRNA. *Nucleic Acids Res.*, **27**, 2063–2071.
17. Müller,F., Döring,T., Erdemir,T., Greuer,B., Jünke,N., Osswald,M., Rinke-Appel,L., Stade,K., Thamm,S. and Brimacombe,R. (1995) Getting closer to an understanding of the three-dimensional structure of ribosomal RNA. *Biochem. Cell Biol.*, **73**, 767–773.
18. Sommer,I. and Brimacombe,R. (2000) Methods for refining interactively established models of ribosomal RNA towards a physico-chemically plausible structure. *J. Comput. Chem.*, in press.