

A network of assembly factors is involved in remodeling rRNA elements during preribosome maturation

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The authors inadvertently omitted Woonghee Lee from the list of authors. The corrected author list and affiliations appear above. Revised acknowledgment paragraphs including Woonghee Lee's funding source and contribution appear below.

In addition, the authors noted the omission of details of NMR structure calculation from the Materials and Methods section. The relevant paragraph and the references associated with it are appended below.

The HTML and PDF versions of this article have been corrected. The errors remain only in the print version.

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Author contributions: J. Baßler and E. Hurt designed the research, and J. Baßler, H. Paternoga, M. Thoms, I. Holdermann, S. Granneman, A. Nyarko, S.A. Clark, G. Stier, D. Schraivogel, and M. Kallas performed experiments. J. Baßler, H. Paternoga, and M. Kallas crystallized proteins. I. Holdermann, and I. Sinning determined and analyzed the crystal structures. W. Lee and A. Nyarko solved and refined the structure of ctNsa-C. NMR analysis was done by A. Nyarko, S.A. Clark, and E. Barbar. In vitro binding assays were done by H. Paternoga, and M. Thoms did the Rpl5 experiments. J. Baßler did the sucrose gradient analysis, S. Granneman did the CRAC experiment, and S. Granneman and D. Tollervey analyzed data. J. Baßler, H. Paternoga, M. Thoms, C. Barrio-Garcia, E. Hurt, and R. Beckmann interpreted EM data. J. Baßler and E. Hurt wrote the manuscript.

NMR structure calculation

A list of chemical shift assignments and ¹³C- and ¹⁵N-filtered 3D NOESY data were provided as input to PONDEROSA-C/S (Lee et al., 2014), which with its refinement option solved the initial structure. PONDEROSA-C/S incorporates TALOS-N (Shen and Bax, 2013) for the generation of structural restraints from chemical shifts and CYANA (Güntert, 2004) for structure determination. Next, hydrogen bond constraints were generated by predictions from CYANA, TALOS-N, PSIPRED (Buchan et al., 2013), analysis of NOESY spectra and the initial structure. Analysis was aided by use of Ponderosa Analyzer, NMRFAM-SPARKY (Lee et al., 2015) with the Ponderosa plug-in, and PyMOL. Ponderosa Analyzer was used to validate and refine distance and angle constraints and to export these to the Ponderosa Client for

iterative structure calculations by the Ponderosa Server. Water refinement by XPLOR-NIH (Schwieters et al., 2003) was carried out by the Ponderosa Server. The final structure, as represented by 20 models, was analyzed by the protein structure validation software suite (PSVS; Bhattacharya et al., 2007).

References

- Bhattacharya, A., R. Tejero, and G.T. Montelione. 2007. Evaluating protein structures determined by structural genomics consortia. *Proteins*. 66:778–795. <http://dx.doi.org/10.1002/prot.21165>
- Buchan, D.W., F. Minneci, T.C. Nugent, K. Bryson, and D.T. Jones. 2013. Scalable web services for the PSIPRED Protein Analysis Workbench. *Nucleic Acids Res.* 41:W349–W357. <http://dx.doi.org/10.1093/nar/gkt381>
- Lee, W., J.L. Stark, and J.L. Markley. 2014. PONDEROSA-C/S: client-server based software package for automated protein 3D structure determination. *J. Biomol. NMR*. 60:73–75. <http://dx.doi.org/10.1007/s10858-014-9855-x>
- Lee, W., M. Tonelli, and J.L. Markley. 2015. NMRFAM-SPARKY: enhanced software for biomolecular NMR spectroscopy. *Bioinformatics*. 31:1325–1327. <http://dx.doi.org/10.1093/bioinformatics/btu830>
- Schwieters, C.D., J.J. Kuszewski, N. Tjandra, and G.M. Clore. 2003. The Xplor-NIH NMR molecular structure determination package. *J. Magn. Reson.* 160:65–73. [http://dx.doi.org/10.1016/S1090-7807\(02\)00014-9](http://dx.doi.org/10.1016/S1090-7807(02)00014-9)
- Shen, Y., and A. Bax. 2013. Protein backbone and sidechain torsion angles predicted from NMR chemical shifts using artificial neural networks. *J. Biomol. NMR*. 56:227–241. <http://dx.doi.org/10.1007/s10858-013-9741-y>