Jiro Kondo,^{a*} Anne-Catherine Dock-Bregeon,^b Dagmar K. Willkomm,^c Roland K. Hartmann^d and Eric Westhof^e

^aDepartment of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, Tokyo 102-8554, Japan, ^bDepartement de Biologie et Génomique Structurales, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Université de Strasbourg CNRS, 67404 Illkirch, France, ^cInstitut für Medizinische Mikrobiologie und Hygiene, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany, ^dInstitut für Pharmazeutische Chemie, Philipps Universität Marburg, Marbacher Weg 6, 35037 Marburg, Germany, and ^eArchitecture et Réactivité de l'ARN, Université de Strasbourg, Institut de Biologie Moléculaire et Cellulaire, CNRS, 15 rue René Descartes, 67084 Strasbourg, France

Correspondence e-mail: j.kondo@sophia.ac.jp

Received 2 April 2013

Accepted 13 May 2013

PDB Reference: A-form DNA, 4jrt

Structure of an A-form RNA duplex obtained by degradation of 6S RNA in a crystallization droplet

In the course of a crystallographic study of a 132 nt variant of *Aquifex aeolicus* 6S RNA, a crystal structure of an A-form RNA duplex containing 12 base pairs was solved at a resolution of 2.6 Å. In fact, the RNA duplex is part of the 6S RNA and was obtained by accidental but precise degradation of the 6S RNA in a crystallization droplet. 6S RNA degradation was confirmed by microscopic observation of crystals and gel electrophoresis of crystallization droplets. The RNA oligomers obtained form regular A-form duplexes containing three GoU wobble-type base pairs, one of which engages in intermolecular contacts through a ribose-zipper motif at the crystal-packing interface.

1. Introduction

Crystal structures of large RNAs such as ribosomal subunits of bacteria (Wimberly *et al.*, 2000; Ban *et al.*, 2000; Yusupov *et al.*, 2001; Harms *et al.*, 2001) and eukaryotes (Ben-Shem *et al.*, 2010; Rabl *et al.*, 2011; Klinge *et al.*, 2011), RNase P (Torres-Larios *et al.*, 2005; Reiter *et al.*, 2010), group I (Adams *et al.*, 2004; Golden *et al.*, 2005; Stahley & Strobel, 2005) and group II (Toor *et al.*, 2008) introns, ribozymes (Scott, 2007) and riboswitches (Edwards *et al.*, 2007) have been solved in recent years. However, the crystallization of large RNAs is still a challenging task. The largest problem, shared not only by crystallographers but also by most RNA scientists, is the degradation of RNA by ribonucleases (RNases) in the environment and by spontaneous cleavage resulting from alkaline or metal-ion-induced hydrolysis.

In the present study, we aimed to solve the crystal structure of 6S RNA, which is a highly abundant bacterial noncoding RNA that regulates transcription through interaction with RNA polymerase (Wassarman & Storz, 2000; Willkomm & Hartmann, 2005; Wassarman, 2007). We successfully obtained large single crystals of a 132 nt variant of 6S RNA from *Aquifex aeolicus*. However, these were subject to damage by X-ray radiation and only diffracted to low resolution. Two months after setting up crystallization droplets, we obtained a different type of crystal that diffracted to 2.6 Å resolution. Surprisingly, the crystal was composed of a 12 base-pair (bp) RNA duplex which is part of the terminal stem of the 6S RNA variant analyzed here. This means that the 6S RNA was degraded in the crystallization droplet. In native *A. aeolicus* 6S RNA, the 12 bp RNA duplex represents an internal helical segment that is extended by another 10 bp towards the 5' and 3' ends (see Fig. 1 for details).

Here, we confirm the 6S RNA degradation process by microscopy and gel electrophoresis and detail the crystal structure of the A-form RNA duplex released as a consequence of the degradation.

2. Materials and methods

2.1. Crystallization

A major portion of the 6S RNA gene from *A. aeolicus* (132 nucleotides; Willkomm *et al.*, 2005; see Fig. 1 for details) was cloned into plasmid pUC19 under control of a T7 promoter and followed by an HDV ribozyme (Mörl *et al.*, 2005). The vector was linearized with *Bam*HI before *in vitro* transcription by T7 RNA polymerase. After

Figure 1

Proposed secondary structure of the major portion (132 nt) of *A. aeolicus* 6S RNA used in this study. Compared with native *A. aeolicus* 6S RNA (Willkomm *et al.*, 2005), the 5'-terminal 19 nt and the 3'-terminal 15 nt were replaced with two G–C base pairs in this variant. The 12 bp RNA duplex obtained by degradation of the 132 nt RNA and crystallized in this study is highlighted by the red box.

Figure 2

Microscopic observations of a crystallization droplet incubated at 310 K after (a) 3 d, (b) one week and (c) three months.

transcription and ribozyme self-cleavage, the transcribed RNA was purified by 10%(w/v) polyacrylamide gel electrophoresis (PAGE) under denaturing conditions (8 M urea), desalted by ultrafiltration and dried by centrifugal concentration. The RNA pellet was dissolved in DEPC-treated RNase-free water and stored at 193 K.

Screening of crystallization conditions was performed by the sitting-drop vapour-diffusion method. Crystallization droplets were prepared by mixing 0.1 µl 0.5 mg ml⁻¹ RNA solution and 0.1 µl of crystallization solutions from several homemade and commercial screening kits using a Mosquito crystallization robot (TTP LabTech, Melbourn, England). Crystallization plates were incubated at 293 and 310 K.

Plate-shaped crystals (0.2 × 0.1 × 0.01 mm in size) grew in 1 d at 293 K and in 3 d at 310 K in conditions consisting of 50 mM sodium cacodylate pH 7.0, 700–1500 mM lithium acetate, 25–30%(v/v) PEG 3350 (see Fig. 2). However, the crystals started to degrade within the next 3 d at 310 K and within the next week at 293 K and completely dissolved within about the next month at both temperatures. Therefore, the plate-shaped crystals were mounted in nylon Cryo-Loops (Hampton Research, Aliso Viejo, California, USA) and stored in liquid nitrogen immediately after appearing in crystallization droplets.

Two months after the 6S RNA crystals had disappeared from the crystallization droplets, rod-shaped crystals (0.3 × 0.05 × 0.02 mm in size) were obtained only at 310 K (see Fig. 2). The crystals were physically much stronger than the plate-shaped crystals mentioned above. The crystals were scooped up using cryoloops and flash-cooled in liquid nitrogen before conducting the X-ray experiment.

2.2. Electrophoresis

The size of the RNA fragments in the crystallization droplets was analyzed by 10% denaturing PAGE. The 6S RNA fragment stored for crystallization experiments (132 nt) and an RNA oligonucleotide (22 nt) were used as size markers. Crystallization droplets incubated at 310 and 293 K for three months were mixed with a loading buffer consisting of 8 M urea, 0.25%(w/v) bromophenol blue, 0.25%(w/v) xylene cyanol and were applied onto the gel. Gel electrophoresis was carried out at room temperature and 100 V. The gel was stained with 0.05%(w/v) toluidine blue O.

2.3. Data collection

X-ray data were collected from the plate-shaped and rod-shaped crystals at 100 K using synchrotron radiation on beamlines ID29 and ID23-1, respectively, of the European Synchrotron Radiation Facility (ESRF), Grenoble, France. Diffraction images were recorded on a CCD detector using 1° oscillation and 1 s exposure time per frame. The plate-shaped crystal diffracted to less than 4 Å resolution. Radiation damage of the crystal during the X-ray experiment was clearly visible, as shown in Supplementary Fig. S1¹. The rod-shaped crystal diffracted much better (2.6 Å resolution) than the plate-shaped crystal without suffering serious radiation damage.

The data sets were processed with *CrystalClear* (Rigaku Americas, The Woodlands, Texas, USA). The plate-shaped and rod-shaped

¹ Supplementary material has been deposited in the IUCr electronic archive (Reference: TZ5032).

Table 1

Crystal data and data-collection and structure-refinement statistics.

Values in parentheses are for the outer shell.

	Plate-shaped crystal	Rod-shaped crystal
Crystal data		
Space group	<i>C</i> 2	<i>P</i> 4 ₃
Unit-cell parameters (Å)	<i>a</i> = 151.9, <i>b</i> = 48.9, <i>c</i> = 60.2	<i>a</i> = <i>b</i> = 29.3, <i>c</i> = 89.8
Unit-cell volume (Å ³)	402141	77213
Asymmetric unit volume (Å ³)	100535	19303
<i>Z</i> †	1 [6S RNA]	1 [RNA duplex]
Solvent content (%)	51.1	53.4
Data collection		
Beamline	ID29, ESRF	ID23-1, ERSF
Wavelength (Å)	1.0	1.0723
Resolution (Å)	35.7–5.0 (5.2–5.0)	20.7–2.6 (2.7–2.6)
Unique reflections	1264	2330
Completeness (%)	69.7 (74.9)	99.6 (100.0)
<i>R</i> _{merge} ‡ (%)	6.7 (42.5)	9.0 (39.7)
Multiplicity	2.3 (2.2)	7.2 (7.5)
Structure refinement		
Resolution range (Å)		20.7–2.6
Used reflections		2330
<i>R</i> factor§ (%)		18.5
<i>R</i> _{free} ¶ (%)		22.8
No. of RNA atoms		517
No. of waters		23
R.m.s.d., bond lengths (Å)		0.006
R.m.s.d., bond angles (°)		1.2

† Estimated number of 6S RNAs (132 nt) or RNA duplexes (12 bp) in the asymmetric unit. ‡ $R_{\text{merge}} = 100 \times \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$. § *R* factor = $100 \times \sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|$, where $|F_{\text{obs}}|$ and $|F_{\text{calc}}|$ are optimally scaled observed and calculated structure-factor amplitudes, respectively. ¶ Calculated using a random set containing 10% of observations.

crystals belonged to space groups *C*2 and *P*4₃, respectively. It was estimated from the unit-cell volume that there was one 6S RNA in the asymmetric unit of the plate-shaped *C*2 crystal. However, the asymmetric unit of the rod-shaped *P*4₃ crystal was too small to contain one 6S RNA, suggesting that the crystal was made of some other RNA molecule(s) in the crystallization droplet.

The obtained intensities were converted to structure-factor amplitudes using the program *TRUNCATE* from the *CCP4* suite (Winn *et al.*, 2011). The statistics of data collection and the crystal data are summarized in Table 1.

2.4. Structure determination and refinement of the rod-shaped crystal

The rod-shaped *P*4₃ crystal had the same space group and similar unit-cell parameters as the crystal of *Thermus flavus* 5S rRNA domain A (Betzel *et al.*, 1994). Therefore, the crystal structure of the 5S rRNA domain A (PDB entry 353d) was used as a starting model for phase determination with the molecular-replacement program *AMoRe* (Navaza, 1994). The molecular structure was constructed and manipulated with *Coot* (Emsley & Cowtan, 2004; Emsley *et al.*, 2010). The atomic parameters were refined using *CNS* by a combination of simulated annealing, crystallographic conjugate-gradient minimization refinement and *B*-factor refinement (Brünger, 1992; Brünger *et al.*, 1998). The statistics of the structure refinement are summarized in Table 1. Molecular drawings were made using *PyMOL* (DeLano, 2002). The local base-pair parameters and pseudo-rotation phase angles of ribose rings given in Supplementary Table S1 were calculated using the program *3DNA* (Lu & Olson, 2003; Olson *et al.*, 2001). The atomic coordinates and experimental data have been deposited in the Protein Data Bank (PDB) with code 4jrt.

3. Results and discussion

3.1. Degradation of 6S RNA in crystallization droplets

Gel electrophoresis confirmed that the 6S RNA was degraded in the crystallization droplets after incubation for three months at 310 and 293 K (see lanes 3 and 4 of Fig. 3), despite the fact that the 6S RNA was carefully prepared to avoid RNase contamination (see the single band in lane 2 of Fig. 3). For the crystallization droplet incubated at 293 K a ladder-like pattern was observed, but a major band corresponding to intact 6S RNA (132 nt) was still present. However, in the crystallization droplet incubated at 310 K the 6S RNA was completely degraded and only a few bands shorter than 22 nt were observed. Since the rod-shaped crystal was only obtained after three months at 310 K, the crystal was anticipated to consist of one or some of these short RNA fragments. RNase contamination of sitting-drop crystallization plates and/or crystallization buffers is difficult to exclude completely, but RNase degradation usually leads to complete conversion of the RNA to nucleotides. However, the observed crystals instead suggested that site-specific RNA cleavage corresponding to local alkaline hydrolysis had occurred. This has previously been observed and discussed for crystals of yeast tRNA^{Asp} (Moras *et al.*, 1985) and is now regularly exploited by use of the ‘in-line probing’ method (Mandal & Breaker, 2004).

3.2. Structure of the terminal stem of the 132 nt 6S RNA variant

The asymmetric unit of the rod-shaped crystal consists of a short RNA duplex obtained as expected from the degradation pattern (see Fig. 3). Electron densities confirmed that the RNA duplex represents the 12 terminal base pairs of the 6S RNA variant shown in Fig. 1. This suggests that this terminal stem region is more resistant to degradation than the other parts of the molecule. The observed structure also results from the fact that among the multiple RNA fragments evident from gel electrophoresis, the particular sequence of this helical RNA

Figure 3

Denaturing 10%(w/v) polyacrylamide gel electrophoresis. A 22 nt RNA oligonucleotide (lane 1) and the intact 132 nt 6S RNA transcript (lane 2) were used as size markers.

fragment has a propensity to crystallize by forming favourable crystal-packing contacts (as described below).

The secondary and the three-dimensional structures of the RNA duplex are shown in Figs. 4(a) and 4(b), respectively. All residues adopt C3'-*endo* sugar pucker, suggesting that the RNA duplex is in the canonical A-form conformation, which is supported by the local base-pair parameters (see Supplementary Table S1).

In the duplex, nine canonical Watson–Crick base pairs (eight G=C and one A=U) are formed. In addition, three wobble-type (*cis* Watson–Crick) GoU base pairs are observed, each forming two hydrogen bonds: N1(G)–H···O2(U) and O6(G)···H–N3(U). Water molecules form bridges between O4(U₄) and O6(G₂₁) and between O6(G₅) and O4(U₂₀) to stabilize the tandem wobble base pairs (see Fig. 4c). The observed motif of tandem GoU base pairs is

the most frequently found in ribosomal RNA sequences (noted as motif I in Masquida & Westhof, 2000). Accordingly, the inclination and twist angles between the tandem U₄oG₂₁ and G₅oU₂₀ base pairs are higher and lower than the average values, respectively (see Supplementary Table 1).

The RNA duplex is similar to the *T. flavus* 5S rRNA domain A, which was used as a starting model for molecular replacement (Betzel *et al.*, 1994; PDB entry 353d), and to an RNA duplex containing tandem UoU base pairs (Baeyens *et al.*, 1995; PDB entry 205d). As mentioned in §2, the former structure (353d) has the same space group as and similar unit-cell parameters to the present RNA duplex. On the other hand, the structure 205d has similar unit-cell parameters but belongs to a different space group, *P*₄₁. Superimpositions of the phosphate-ribose backbones between the present structure and 353d

Figure 4

Secondary structure (a) and crystal structure (b) of an A-form RNA duplex obtained by degradation of the 6S RNA variant in a crystallization droplet and hydrogen-bonding interactions of the three wobble-type GoU base pairs observed in the duplex (c), including two water molecules (red spheres).

Figure 5

View of the crystal-packing interactions observed in the rod-shaped *P*₄₃ crystal. The ribose-zipper motifs observed between two symmetry-related molecules (coloured red and green) are highlighted in (b). Detailed views are shown in Figs. 6(b) and 6(c).

or 205d give root-mean-square deviations of 1.1 and 1.5 Å, respectively (see Supplementary Fig. S2).

3.3. Crystal packing

The intermolecular packing observed in the rod-shaped $P4_3$ crystal is shown in Fig. 5. The A-form RNA duplexes pack head to tail and form endless helices along the crystallographic a and b axes. The duplexes also make perpendicular side-by-side packing interactions along the c axis according to the left-handed fourfold screw (4_3) symmetry operation. The 5S rRNA domain A and the RNA duplex with tandem UoU base pairs pack in a similar but slightly different manner in their $P4_3$ and $P4_1$ crystals, respectively (see Supplementary Fig. S3).

3.4. Ribose-zipper motifs observed at the crystal-packing interface

The RNA duplexes interact *via* ribose-zipper motifs at the perpendicular side-by-side packing interface (see Fig. 6). Three canonical Watson–Crick base pairs, $G_3=C_{22}$, $G_8=C_{17}$ and $C_9=G_{16}$, as well as a wobble-type U_4G_{21} base pair, are involved in the interaction. Between $C_{22}=G_3$ and $G_8=C_{17}$, a symmetrical G_3O_8 sugar edge–sugar edge base pair with two hydrogen bonds between $N2-H$ and $O2'$ (one of them is weak with a long distance) is formed (see Fig. 6*b*). Between $G_{21}O_4$ and $C_9=G_{16}$, five hydrogen bonds are formed: $N2(G_{16})-H \cdots O3'(U_4)$, $O2(C_9) \cdots H-O2'(U_4)$, $O2'(C_9)-H \cdots O2'(U_4)$ [or $O2'(C_9)-H \cdots O2(U_4)$], $O2'(C_9) \cdots H-N2(G_{21})$ and $O3'(C_9) \cdots H-N2(G_{21})$ (see Fig. 6*c*). The ribose-zipper motif between GoU and C=G base pairs in which U and C are at the internal positions is the most frequently observed geometry in crystal structures of small and large ribosomal subunits (Gagnon & Steinberg, 2002; Mokdad *et al.*, 2006). As seen from the geometry of the interaction, the G_{21} residue shifts toward the shallow/minor groove and makes two hydrogen bonds to O atoms of the ribose (see Fig. 6*c*). The wobble-type GoU base pair is known to be favourable for the formation of such a ribose-zipper motif between two *cis* Watson–

Crick base pairs (Gagnon & Steinberg, 2002; Mokdad *et al.*, 2006). To illustrate this, the wobble-type GoC^+ (see the caption of Supplementary Fig. S4) and GoU pairs make ribose-zipper motifs in the two similar crystals mentioned above (Supplementary Figs. S4 and S5).

4. Conclusions

RNA–RNA interactions are central to the intramolecular contacts leading to the folding and final architecture of large RNAs as well as to the formation of the regular intermolecular contacts necessary for precise packing of RNA molecules in crystal lattices. The main types of RNA–RNA contacts, in decreasing order of sequence requirements and specificity (Fritsch & Westhof, 2010), are (i) Watson–Crick pairs between complementary strands, (ii) the GNRA-tetraloop families binding to specific Watson–Crick base-paired helical regions or a complex 11 nt motif (Cate *et al.*, 1996), (iii) A-minor contacts (Nissen *et al.*, 2001) and (iv) the various classes of ribose zippers (Cate *et al.*, 1996; Tamura & Holbrook, 2002). Except for the first case, all of the interactions involve at least one shallow minor-groove side and, in the ribose zippers, the shallow minor grooves on the two interacting RNAs. For each of those types of interactions, examples exist in which such contacts mediate crystal packing. Complementary Watson–Crick base pairing occurs between anticodon triplets in the crystal contacts of yeast tRNA–Asp (Westhof *et al.*, 1985). GNRA tetraloops contact Watson–Crick base pairs, which was first shown in the crystal structure of a hammerhead ribozyme (Pley *et al.*, 1994). A-minor contacts are the main component of crystal contacts in the structures of complexes between aminoglycosides and fragments of the A site (François *et al.*, 2005; Lescoute & Westhof, 2006). The present crystal structure adds a further example of the importance of the packing between roughly perpendicular double-stranded RNA helices *via* the minor grooves of GoU and G=C pairs. Such types of contacts could be used in several biological systems, for example in

Figure 6

The ribose-zipper motifs observed at the crystal-packing interface (Fig. 5*b*) are boxed in (a) and detailed views are shown in (b) and (c). The hydrogen bonds involved in the motifs are represented by dashed lines with distances in Å.

the packing of helices during the encapsulation of genomic RNAs in viruses.

We thank the European Synchrotron Radiation Facility and acknowledge the staff of beamlines ID23-1 and ID29. Funding from the Deutsche Forschungsgemeinschaft (HA-1672/16-1) to DKW and RKH is acknowledged.

References

- Adams, P. L., Stahley, M. R., Kosek, A. B., Wang, J. & Strobel, S. A. (2004). *Nature (London)*, **430**, 45–50.
- Baeyens, K. J., De Bondt, H. L. & Holbrook, S. R. (1995). *Nature Struct. Biol.* **2**, 56–62.
- Ban, N., Nissen, P., Hansen, J., Moore, P. B. & Steitz, T. A. (2000). *Science*, **289**, 905–920.
- Ben-Shem, A., Jenner, L., Yusupova, G. & Yusupov, M. (2010). *Science*, **330**, 1203–1209.
- Betz, C., Lorenz, S., Fürste, J. P., Bald, R., Zhang, M., Schneider, T. R., Wilson, K. S. & Erdmann, V. A. (1994). *FEBS Lett.* **351**, 159–164.
- Brünger, A. T. (1992). *Nature (London)*, **355**, 473–475.
- Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst. D* **54**, 905–921.
- Cate, J. H., Gooding, A. R., Podell, E., Zhou, K., Golden, B. L., Kundrot, C. E., Cech, T. R. & Doudna, J. A. (1996). *Science*, **273**, 1678–1685.
- DeLano, W. L. (2002). *PyMOL*. <http://www.pymol.org>.
- Edwards, T. E., Klein, D. J. & Ferré-D'Amaré, A. R. (2007). *Curr. Opin. Struct. Biol.* **17**, 273–279.
- Emsley, P. & Cowtan, K. (2004). *Acta Cryst. D* **60**, 2126–2132.
- Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst. D* **66**, 486–501.
- François, B., Russell, R. J., Murray, J. B., Aboul-ela, F., Masquida, B., Vicens, Q. & Westhof, E. (2005). *Nucleic Acids Res.* **33**, 5677–5690.
- Fritsch, V. & Westhof, E. (2010). *The Chemical Biology of Nucleic Acids*, edited by G. Mayer, pp. 141–174. Chichester: John Wiley & Sons.
- Gagnon, M. G. & Steinberg, S. V. (2002). *RNA*, **8**, 873–877.
- Golden, B. L., Kim, H. & Chase, E. (2005). *Nature Struct. Mol. Biol.* **12**, 82–89.
- Harms, J., Schlutzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F. & Yonath, A. (2001). *Cell*, **107**, 679–688.
- Klinge, S., Voigts-Hoffmann, F., Leibundgut, M., Arpagaus, S. & Ban, N. (2011). *Science*, **334**, 941–948.
- Lescoute, A. & Westhof, E. (2006). *Biochimie*, **88**, 993–999.
- Lu, X.-J. & Olson, W. K. (2003). *Nucleic Acids Res.* **31**, 5108–5121.
- Mandal, M. & Breaker, R. R. (2004). *Nature Rev. Mol. Cell Biol.* **5**, 451–463.
- Masquida, B. & Westhof, E. (2000). *RNA*, **6**, 9–15.
- Mokdad, A., Krasovska, M. V., Sponer, J. & Leontis, N. B. (2006). *Nucleic Acids Res.* **34**, 1326–1341.
- Moras, D., Dock, A. C., Dumas, P., Westhof, E., Romby, P., Ebel, J. P. & Giegé, R. (1985). *J. Biomol. Struct. Dyn.* **3**, 479–493.
- Mörl, M., Lizano, E., Willkomm, D. K. & Hartmann, R. K. (2005). *Handbook of RNA Biochemistry*, edited by R. K. Hartmann, A. Bindereif, A. Schön & E. Westhof, pp. 22–35. Weinheim: Wiley-VCH.
- Navaza, J. (1994). *Acta Cryst. A* **50**, 157–163.
- Nissen, P., Ippolito, J. A., Ban, N., Moore, P. B. & Steitz, T. A. (2001). *Proc. Natl Acad. Sci. USA*, **98**, 4899–4903.
- Olson, W. K. *et al.* (2001). *J. Mol. Biol.* **313**, 229–237.
- Pley, H. W., Flaherty, K. M. & McKay, D. B. (1994). *Nature (London)*, **372**, 68–74.
- Rabl, J., Leibundgut, M., Ataide, S. F., Haag, A. & Ban, N. (2011). *Science*, **331**, 730–736.
- Reiter, N. J., Osterman, A., Torres-Larios, A., Swinger, K. K., Pan, T. & Mondragón, A. (2010). *Nature (London)*, **468**, 784–789.
- Scott, W. G. (2007). *Curr. Opin. Struct. Biol.* **17**, 280–286.
- Stahley, M. R. & Strobel, S. A. (2005). *Science*, **309**, 1587–1590.
- Tamura, M. & Holbrook, S. R. (2002). *J. Mol. Biol.* **320**, 455–474.
- Toor, N., Keating, K. S., Taylor, S. D. & Pyle, A. M. (2008). *Science*, **320**, 77–82.
- Torres-Larios, A., Swinger, K. K., Krasilnikov, A. S., Pan, T. & Mondragón, A. (2005). *Nature (London)*, **437**, 584–587.
- Wassarman, K. M. (2007). *Curr. Opin. Microbiol.* **10**, 164–168.
- Wassarman, K. M. & Storz, G. (2000). *Cell*, **101**, 613–623.
- Westhof, E., Dumas, P. & Moras, D. (1985). *J. Mol. Biol.* **184**, 119–145.
- Willkomm, D. K. & Hartmann, R. K. (2005). *Biol. Chem.* **386**, 1273–1277.
- Willkomm, D. K., Minnerup, J., Hüttenhofer, A. & Hartmann, R. K. (2005). *Nucleic Acids Res.* **33**, 1949–1960.
- Wimberly, B. T., Brodersen, D. E., Clemons, W. M., Morgan-Warren, R. J., Carter, A. P., Vornrhein, C., Hartsch, T. & Ramakrishnan, V. (2000). *Nature (London)*, **407**, 327–339.
- Winn, M. D. *et al.* (2011). *Acta Cryst. D* **67**, 235–242.
- Yusupov, M. M., Yusupova, G. Z., Baucom, A., Lieberman, K., Earnest, T. N., Cate, J. H. & Noller, H. F. (2001). *Science*, **292**, 883–896.