Crystal structure of the human heterogeneous ribonucleoprotein A18 RNA-recognition motif

Katherine Coburn,^{a‡} Zephany Melville,^{a‡} Ehson Aligholizadeh,^a Braden M. Roth,^a Kristen M. Varney,^a France Carrier,^b Edwin Pozharski^a and David J. Weber^{a*}

^aDepartment of Biochemistry and Molecular Biology, Center for Biomolecular Therapeutics, University of Maryland School of Medicine, 108 North Greene Street, Baltimore, MD 21201, USA, and ^bDepartment of Radiation Oncology, Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine, 655 West Baltimore Street, Baltimore, MD 21201, USA. *Correspondence e-mail: dweber@som.umaryland.edu

Received 3 November 2016

Accepted 2 March 2017

Edited by R. J. Read, University of Cambridge, England

‡ These authors contributed equally to this work.

Keywords: crystal structure; RNA-binding protein; heterogeneous ribonucleoprotein A18; hnRNP A18; RNA-recognition motif; tumor growth.

PDB reference: RNA-recognition motif of hnRNP A18, 5tbx

Supporting information: this article has supporting information at journals.iucr.org/f

The heterogeneous ribonucleoprotein A18 (hnRNP A18) is upregulated in hypoxic regions of various solid tumors and promotes tumor growth *via* the coordination of mRNA transcripts associated with pro-survival genes. Thus, hnRNP A18 represents an important therapeutic target in tumor cells. Presented here is the first X-ray crystal structure to be reported for the RNA-recognition motif of hnRNP A18. By comparing this structure with those of homologous RNA-binding proteins (*i.e.* hnRNP A1), three residues on one face of an antiparallel β -sheet (Arg48, Phe50 and Phe52) and one residue in an unstructured loop (Arg41) were identified as likely to be involved in protein–nucleic acid interactions. This structure helps to serve as a foundation for biophysical studies of this RNA-binding protein and structure-based drug-design efforts for targeting hnRNP A18 in cancer, such as malignant melanoma, where hnRNP A18 levels are elevated and contribute to disease progression.

1. Introduction

Heterogeneous ribonucleoprotein A18 (hnRNP A18) is an RNA-binding protein that is expressed in a variety of cell and tissue types, and its contribution to translation initiation is induced by cellular stresses such as UV radiation, hypoxia and cold shock (Sheikh *et al.*, 1997; Nishiyama *et al.*, 1997). hnRNP A18 is thus also known as cold-inducible RNA-binding protein. hnRNP A18 is located predominantly in the nucleus under normal conditions, but under cellular stress hnRNP A18 is phosphorylated by kinases such as GSK-3 β and/or CK2 (Yang & Carrier, 2001; Yang *et al.*, 2010). Phosphorylated hnRNP A18 translocates from the nucleus to the cytoplasm (Yang *et al.*, 2010). In the cytoplasm, hnRNP A18 binds mRNA, promoting the translation of several pro-survival gene products such as thioredoxin (Yang *et al.*, 2006), DNA damage-repair protein RPA (Yang & Carrier, 2001) and telomerase (Zhang *et al.*, 2016). The binding of hnRNP A18 to mRNA is mediated in part through a defined RNA-recognition motif (RRM), which is a domain of approximately 90 amino-acid residues that binds to single-stranded RNA (Dreyfuss *et al.*, 1988; Sachs *et al.*, 1987) including an mRNA consensus sequence that is found specifically in hnRNP A18 target genes (Yang *et al.*, 2010). It is the details of the hnRNP A18–mRNA complex that are of particular interest, as such information will contribute to a more complete mechanistic understanding of how hnRNP A18 regulates translation initiation for specific target genes in both normal and transformed cells.

Table 1

Data collection and processing.

Values in parentheses are for the outer shell.

PDB code	5tbx
Radiation source	Beamline 24-ID-E, APS
Wavelength (Å)	0.979
Temperature (K)	100
Detector	ADSC Quantum 315
Space group	$P2_12_12_1$
a, b, c (Å)	41.5, 56.1, 72.4
Mosaicity (°)	0.26
Resolution range (Å)	44.38–1.77 (1.83–1.77)
Total reflections	147456 (13753)
No. of unique reflections	17166 (1607)
Completeness (%)	99 (99)
Multiplicity	8.6 (8.3)
$\langle I/\sigma(I) \rangle$	13.70 (0.83)
$CC_{1/2}$	0.993 (0.486)
R_{meas}	0.169 (3.189)
$R_{\text{p.i.m.}}$	0.075 (1.778)
Overall B factor from Wilson plot (Å ²)	22.4
No. of reflections, working set	16927 (1602)
No. of reflections, test set	883 (76)
Final R_{cryst}	0.2177 (0.3959)
Final R_{free}	0.2474 (0.4327)
No. of non-H atoms	
Protein	1358
Nonprotein atoms	5
Solvent	124
Total	1487
R.m.s. deviations	
Bonds (Å)	0.013
Angles (°)	1.13
Average B factors (Å ²)	
Overall	34.62
Protein	33.85
Ligands	48.10
Ramachandran plot†	
Most favored (%)	97.7
Allowed (%)	2.3

† Ramachandran statistics are derived from *MolProbity* (Chen *et al.*, 2010).

Artero-Castro and coworkers recently showed hnRNP A18 to be upregulated in the cytosol of human melanoma, breast, colon and prostate cancer cells (Artero-Castro *et al.*, 2009), and immunohistochemistry data demonstrated that hnRNP A18 is overexpressed in solid tumors compared with normal tissue from the same patients (Chang *et al.*, 2016). This is congruent with most solid tumors developing hypoxic micro-environments at their center, having outgrown the nearby vasculature (Harris, 2002). Upregulation of hnRNP A18 is advantageous for cancerous cells owing to the stabilization of proliferation-associated mRNA harboring the hnRNP A18 signature motif in their 3'-UTRs. Importantly, when hnRNP A18 protein levels are reduced *via* siRNA approaches, tumor growth is decreased significantly in mouse models (Chang *et al.*, 2016). As such, hnRNP A18 represents a novel therapeutic target for selectively blocking translation initiation for several growth-promoting transcripts in cancer.

Targeting translation initiation *via* blocking hnRNP A18 provides a therapeutic advantage for treating cancer. In particular, normal cells express hnRNP A18 at very low levels and primarily in the nucleus, so its activation *via* stress-activated kinases under normal conditions is minimal (Chang *et al.*, 2016), whereas in cancer cells hypoxia and other stress

responses lead to hnRNP A18 overproduction and a cascade of pro-survival activities from its targeted gene products. Thus, the development of small-molecule inhibitors for hnRNP A18 will abrogate this pro-survival cascade that occurs in many human cancers. Thus, it is critically important that specific structure–function relationships are delineated for hnRNP A18 and that structure-based drug-design approaches take advantage of these data for drug design using a combination of X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy data (PDB entry 1x5s; RIKEN Structural Genomics/Proteomics Initiative, unpublished work). Owing to the conserved nature of the RRM domain, rational design of small-molecule inhibitors will have to take advantage of the unique hnRNP A18 RRM–RNA interactions to create inhibitors specific for targeting hnRNP A18 (*i.e. versus* other RNA-binding proteins). Towards these goals, the crystal structure of the hnRNP A18 RRM reported here provides pertinent atomic level details regarding backbone and side-chain positioning for residues throughout the RRM domain and represents a major step towards answering mechanistic questions regarding hnRNP A18-dependent activation of translation initiation. Furthermore, comparisons of the hnRNP A18 RRM structure with an analogous domain of the human heterogeneous ribonucleoprotein A1 (hnRNP A1) provide pertinent information regarding the mechanism by which hnRNP A18 interacts with mRNA *via* three residues (Arg48, Phe50 and Phe52) on one face of a β -sheet and one residue (Arg41) in an unstructured loop identified in this structure.

2. Methods

2.1. Materials

Chromatography columns and resins were obtained from GE Healthcare Life Sciences. All other chemical reagents used were ACS grade or higher and were purchased from Sigma–Aldrich unless stated otherwise.

2.2. Expression and purification of human heterogeneous ribonucleoprotein A18 RNA-recognition motif (RRM)

Wild-type human hnRNP A18 (residues 1–172) contains an RRM (residues 1–92) and an intrinsically disordered domain (IDD; residues 93–172) that contains the GSK-3 β and CK2 protein kinase substrate sites of hnRNP A18 (residues 138–172). For the structural studies reported here, a 93-amino-acid (~10.1 kDa) truncated version of human hnRNP A18 was produced that encompasses a single non-native glycine (Gly1; G1) followed by the first 92 residues of hnRNP A18. This construct represents the structured RRM and is hereafter termed hnRNP A18 RRM. The hnRNP A18 RRM construct was cloned into the *Escherichia coli* expression plasmid pHGK-IF (unpublished) in-frame and downstream of a *Tobacco etch virus* (TEV) protease-cleavable N-terminally 6 $\times$ His-tagged GB1 (protein G, B1 domain) fusion protein. pHGK-A18 RRM was transformed into *E. coli* BL21(DE3) cells and a single colony was selected for expression. The cells

were grown in 1 l M9 minimal medium (Sambrook & Russell, 2001) with ^{15}N -labeled (>99%) ammonium chloride as the sole nitrogen source at 37°C until the A_{600} reached 0.8. The incubation temperature was reduced to 18°C, His₆GB1-A18 RRM expression was induced by the addition of 0.3 mM IPTG (isopropyl β -D-1-thiogalactopyranoside) and the cells were grown for an additional 16 h. The cells were pelleted by centrifugation at 10 000g for 20 min and were resuspended in denaturing buffer (buffer A; 20 mM Tris pH 7.4, 500 mM NaCl, 5 mM imidazole, 6 M urea). The cells were lysed by sonication and centrifuged at 18 000g for 30 min to pellet the cell debris. The cleared lysate was applied onto a 5 ml HisTrap FF column (GE Healthcare, catalog No. 17-5255-01), which was equilibrated with buffer A and refolded in buffer B (20 mM Tris pH 7.4, 500 mM NaCl, 5 mM imidazole) applied as a linear gradient over 20 column volumes. His₆GB1-A18 RRM was eluted from the column with a linear gradient of buffer C (20 mM Tris pH 7.4, 500 mM NaCl, 500 mM imidazole) over 10 column volumes. The eluted fractions were analyzed by SDS-PAGE and fractions containing GB1-A18 RRM were simultaneously treated with His-tagged TEV protease to liberate A18 RRM from the His₆GB1 fusion partner and buffer-exchanged into buffer B *via* overnight dialysis. The sample was applied onto a HisTrap HP column (GE Healthcare, catalog No. 17-5247-01) in buffer A and the flowthrough containing purified A18 RRM was collected. The protein was exchanged into ultrapure water, concentrated, and quantified by absorbance at 280 nm. Approximately 30 mg of [^{15}N]-A18 RRM was typically purified from 1 l M9 medium and its identity was confirmed using NMR (^1H - ^{15}N TROSY) and mass-spectrometric analyses.

2.3. Crystallography

Diffraction-quality crystals of purified human hnRNP A18 RRM were obtained *via* sitting-drop vapor diffusion in 96-well trays (Hampton Research) with 50 μl mother liquor and a 0.75:0.75 μl drop size. The reservoir solution for crystal

formation was 0.2 M sodium acetate trihydrate pH 8.0, 20% (w/v) PEG 3350 (PEG/Ion HT crystallization screen condition C3, Hampton Research). Protein-sample conditions for crystal formation were 12.1 mg ml⁻¹ hnRNP A18 RRM protein in distilled, deionized water. Crystals were flash-cooled in liquid nitrogen for storage prior to data collection with no cryoprotectant used.

Diffraction data were collected on the Northeastern Collaborative Access Team (NE-CAT) 24-ID-E beamline at the Advanced Photon Source (APS), Argonne National Laboratory, Argonne, Illinois, USA. Data were collected at 100 K on an ADSC Quantum 315 3 $\times$ 3 CCD detector with data-collection strategies generated by the NE-CAT *RAPD* automated processing pipeline (<https://rapd.nec.aps.anl.gov/rapd>), which uses *XDS* (Kabsch, 2010a,b), *POINTLESS* (Evans, 2006, 2011) and *AIMLESS* (Evans & Murshudov, 2013) for integration and scaling. The diffraction statistics are summarized in Table 1.

The space group was determined to be $P2_12_12_1$ and the *BALBES* server (Long *et al.*, 2008) was utilized to identify an appropriate search model and to perform molecular replacement with *MOLREP* (Vagin, 1989; Vagin & Teplyakov, 2010). The UP1 region of hnRNP A1 (PDB entry 1u1q; Myers & Shamoo, 2004) was identified as the most appropriate search model. Manual building was performed in *Coot* (Emsley *et al.*, 2010). Refinement of the model was performed using the *phenix.refine* (Afonine *et al.*, 2005) function of the *PHENIX* software suite (Adams *et al.*, 2010). The structure-refinement statistics are summarized in Table 1. The final figures and alignments were generated using *PyMOL* (Schrödinger).

3. Results and discussion

In response to cellular stress, hnRNP A18 is phosphorylated and then translocated from the nucleus to the cytoplasm, where it promotes the translation of pro-survival transcripts harboring its signature motif in their 3'-UTRs (Chang *et al.*, 2016; Yang & Carrier, 2001; Yang *et al.*, 2010). In hypoxic

Figure 1

Ribbon diagram of the hnRNP A18 RRM crystal structure viewed from the front (a) and rear (b) with helices in blue and the β -sheet in green.

cancer cells, downregulation of hnRNP A18 has been shown to significantly decrease tumor proliferation and invasion (Chang *et al.*, 2016). While hnRNP A18 represents an attractive target for the development of pharmacological antagonists, no small-molecule inhibitors have been identified to date. Here, the crystal structure of the hnRNP A18 RRM is presented (Fig. 1) and is solved at 1.77 Å resolution. Fig. 2 illustrates the structural homology of hnRNP A18 to hnRNP A1, and Figs. 3 and 4

highlight the similarities and differences in the RNA-binding sites of hnRNP A18 and hnRNP A1 when the two structures are compared. Specifically, three residues on one face of an antiparallel β -sheet of hnRNP A18 (Arg48, Phe50 and Phe52) and one residue in an unstructured loop (Arg41) were identified as likely to be involved in protein–nucleic acid interactions based on this comparison. These data provide a foundation for studying RNA complexes of hnRNP A18 that could impact on the design of novel inhibitors that target hnRNP A18 in cancers where this protein is expressed at elevated levels.

3.1. Crystal structure of human heterogeneous ribonucleoprotein A18 RRM

Initial attempts to crystallize full-length hnRNP A18 were unsuccessful; however, after the IDD was removed hnRNP A18 RRM readily crystallized using sitting-drop vapor diffusion. X-ray diffraction data were collected and the crystal structure of the hnRNP A18 RRM was solved at 1.77 Å resolution with final R_{cryst} and R_{free} values of 0.2177 and 0.2474, respectively (Table 1, Fig. 1). Two hnRNP A18 RRM molecules were present per asymmetric unit, and the space group, $P2_12_12_1$, was confirmed *via* Zanuda (Lebedev & Isupov, 2014) in the CCP4 software suite (Murshudov *et al.*, 2011; Winn *et al.*, 2011). Consistent with other single-stranded RNA (ssRNA)-binding proteins, the hnRNP A18 RRM has two α -helices and four antiparallel β -strands with a $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$ alignment. The RRM of hnRNP A18 folds into an $\alpha\beta$ sandwich, where the strands of the β -sheet run antiparallel in the order $\beta_4\beta_1\beta_3\beta_2$ and the two α -helices pack against residues on the side of the β -sheet, which is likely not to interact with ssRNA. As found in other RNA-binding proteins, several solvent-exposed aromatic residues reside on the other face of the β -sheet, opposite to the α -helices, and are likely to be

Figure 2
An overlay and alignment of the RRM of hnRNP A18 (green) and the first RRM of hnRNP A1 (gray; PDB entry 1u1q). All atoms align with an r.m.s.d. of 1.23 Å. The second RRM domain of hnRNP A1 has been omitted for clarity.

Figure 3
The electron-density map for the β -sheet of the hnRNP A18 RRM (a); the conserved RNA-binding residues of the hnRNP A18 RRM are highlighted in (b). For clarity, only strands β_1 and β_3 are in green in (a) and the RNA-binding residues are in green in (b). Electron-density maps are displayed at 2.0σ .

involved in the binding of ssRNA (Elson *et al.*, 2000). In fact, when the hnRNP A18 structure was compared with the crystal structure of hnRNP A1 (PDB entry 1u1q; Myers & Shamoo, 2004), the alignment of all atoms had an r.m.s.d. of 1.23 Å (*PyMOL*) and the residues that interact with RNA in hnRNP A1 aligned closely with the predicted RNA-binding residues of hnRNP A18 (Fig. 4).

3.2. Detailed comparison of the RRM of hnRNP A18 and hnRNP A1

Proteins containing RRM have two highly conserved regions that are important for binding single-stranded RNA (Maris *et al.*, 2005), termed ribonucleoprotein domain 1 (RNP1) and ribonucleoprotein domain 2 (RNP2). In a typical RRM, RNP1 is located on β -strand 3 (β_3) and has a consensus sequence defined as (Lys/Arg)-Gly-(Phe/Tyr)-(Gly/Ala)-(Phe/Tyr)-(Val/Ile/Leu)-X-(Phe/Tyr), where *X* is any amino acid (Maris *et al.*, 2005; Dreyfuss *et al.*, 1988; Adam *et al.*, 1986; Swanson *et al.*, 1987). For hnRNP A18, the RNP1 sequence is Arg48-Gly49-Phe50-Gly51-Phe52-Val53-Thr54-Phe55 and, when aligned, differs by only a single amino-acid residue from that found in hnRNP A1, where the last position is a tyrosine residue (*i.e.* Tyr62 in hnRNP A1 *versus* Phe55 in hnRNP A18). For RNP1 of hnRNP A1, the residues involved in binding nucleic acids are Arg55, Phe57 and Phe59, termed positions 1, 3 and 5 of RNP1, respectively (Fig. 4; PDB entry 1u1q). The residues in these positions in hnRNP A18 are Arg48, Phe50 and Phe52, respectively, and it is clear that they also align structurally with positions 1, 3 and 5 of RNP1 in hnRNP A1, as was predicted previously (Myers & Shamoo, 2004). Thus, like Arg55 in hnRNP A1, Arg48 of hnRNP A18 is poised to form a salt bridge with the phosphate of the sugar backbone of the nucleotide; likewise, Phe52 in position 5 of RNP1 in hnRNP A18 could π -stack with a purine base in the hnRNP A18 target RNA, as found for Phe59 in hnRNP A1 (Myers & Shamoo,

2004). Phe50, on the other hand, may form a hydrophobic interaction with the sugar rings of both nucleotides, as is the case for Phe57 of hnRNP A1 (Myers & Shamoo, 2004). Arg41 in hnRNP A18 is also positioned to form a salt bridge with the phosphate of the sugar backbone (Fig. 4), whereas this position is occupied by Pro49 in hnRNP A1 and is therefore a potentially unique RNA interaction for hnRNP A18.

The second RRM consensus sequence (RNP2) is located on β_1 and is defined as (Ile/Val/Leu)-(Phe/Tyr)-(Ile/Val/Leu)-X-Asn-Leu (Dreyfuss *et al.*, 1988). In RNP2 of hnRNP A18 this sequence is Leu9-Phe10-Val11-Gly12-Gly13-Leu14 and differs from the RNP consensus sequence at the fifth position, where hnRNP A18 has a glycine residue rather than an asparagine residue as found in other RRM. Interestingly, hnRNP A1 also has a glycine at position 5 of RNP2, and thus represents the hnRNP protein that most closely matches the hnRNP A18 sequence. Thus, based on the hnRNP A1 structure, Phe10 (position 2) of hnRNP A18 is likely to interact directly with a purine ring *via* aromatic stacking in a manner similar to hnRNP A1 since this residue is structurally conserved when the structures of hnRNP A1 and hnRNP A18 are compared (Fig. 4).

It is important to consider that hnRNP A18 binds to specific mRNA sequences (Yang *et al.*, 2010), yet it retains the global fold of an RRM as found in other RNA-binding proteins in the hnRNP family, including hnRNP A1. While the mRNA-recognition sequence for hnRNP A1 was previously determined to be UAGGGA (Burd & Dreyfuss, 1994), no conserved recognition sequence has been identified for hnRNP A18. However, a 51-nucleotide motif has been identified that is specific for hnRNP A18 but displays U/A-rich regions with low similarity to the hnRNP A1 binding sequence (Yang *et al.*, 2010). This indicates clear RNA-binding specificity between hnRNP A1 and hnRNP A18 despite overall similarities in the conserved RRM. Fig. 2 displays an alignment of the crystal structure of the hnRNP A18 RRM

Figure 4

(a) The interactions between hnRNP A1 (gray) and RNA (yellow) (PDB entry 1u1q). (b) Overlay of hnRNP A1 with hnRNP A18 (green) showing the conserved RNA-binding residues. Arg55 in hnRNP A1 forms a salt bridge with the phosphate of the sugar backbone of the nucleotide. Phe17 and Phe59 form π -stacking interactions with the purine bases of the nucleotide. Phe57 interacts hydrophobically with the sugar rings of both nucleic acids. Arg41 in hnRNP A18 is also poised to form a salt bridge with the phosphate of the sugar backbone. Pro49 instead occupies this same position in hnRNP A1.

determined here with the crystal structure of RRM1 from human hnRNP A1 (PDB entry 1u1q) determined previously (Myers & Shamoo, 2004). The structures of hnRNP A18 and hnRNP A1 align with an r.m.s.d. 1.23 Å for all atoms, revealing that the overall global fold of these two domains is highly conserved, including their RNA-binding motifs, RNP1 and RNP2. Therefore, the differences in the amino-acid sequence for hnRNP A18 and hnRNP A1 (Karlin & Altschul, 1990) at sites outside the RRM s may account for differences in the RNA-binding specificity for these RRM proteins *via* more subtle interactions throughout the protein and/or target RNA. Also of particular interest for further study is the C-terminal IDD of A18 (residues 93–172), which contains several protein kinase substrate sites that are important for transport to the cytoplasm (residues 138–172) and RNA-binding activity (Yang *et al.*, 2006), and that could also provide regions contributing towards RNA-binding specificity.

4. Conclusions

Provided here is the crystal structure of the hnRNP A18 RRM. This structure illustrates a similar global fold to that of the NMR structure of the hnRNP A18 RRM in solution (PDB entry 1x5s) as well as RRM s from other hnRNPs (*i.e.* hnRNP A1). Although few insights into the specificity of the hnRNP A18 RRM–RNA interaction can be derived because of the structural and sequential similarities to hnRNP A1, particularly with regard to the conserved residues in RNP1 and RNP2 (Figs. 3 and 4), this structure can serve as a model for further investigation of full-length hnRNP A18 and of hnRNP A18–RNA complex(es) involving its consensus RNA-binding sequence.

Acknowledgements

Use of the Advanced Photon Source (APS), Argonne National Laboratory is supported by the US Department of Energy (DOE), Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under contract No. DE-AC02-06CH11357. The Northeastern Collaborative Access Team beamlines located at APS are funded by the National Institute of General Medical Sciences from the National Institutes of Health (P41 GM103403). The training of ZM was supported by an NIH grant (NIH T32 AR007592). This work was supported by an NIH grant (NIH R01 CA177981 to FC and DJW).

References

Adam, S. A., Nakagawa, T., Swanson, M. S., Woodruff, T. K. & Dreyfuss, G. (1986). *Mol. Cell. Biol.* **6**, 2932–2943.

- Adams, P. D. *et al.* (2010). *Acta Cryst.* **D66**, 213–221.
- Afonine, P. V., Grosse-Kunstleve, R. W. & Adams, P. D. (2005). *Acta Cryst.* **D61**, 850–855.
- Artero-Castro, A., Callejas, F. B., Castellvi, J., Kondoh, H., Carnero, A., Fernández-Marcos, P. J., Serrano, M., Ramón y Cajal, S. & Leonart, M. E. (2009). *Mol. Cell. Biol.* **29**, 1855–1868.
- Burd, C. G. & Dreyfuss, G. (1994). *EMBO J.* **13**, 1197–1204.
- Chang, E. T., Parekh, P. R., Yang, Q., Nguyen, D. M. & Carrier, F. (2016). *Oncotarget*, **7**, 10578–10593.
- Chen, V. B., Arendall, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S. & Richardson, D. C. (2010). *Acta Cryst.* **D66**, 12–21.
- Dreyfuss, G., Swanson, M. S. & Piñol-Roma, S. (1988). *Trends Biochem. Sci.* **13**, 86–91.
- Elson, D. A., Ryan, H. E., Snow, J. W., Johnson, R. & Arbeit, J. M. (2000). *Cancer Res.* **60**, 6189–6195.
- Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.
- Evans, P. (2006). *Acta Cryst.* **D62**, 72–82.
- Evans, P. R. (2011). *Acta Cryst.* **D67**, 282–292.
- Evans, P. R. & Murshudov, G. N. (2013). *Acta Cryst.* **D69**, 1204–1214.
- Harris, A. L. (2002). *Nature Rev. Cancer*, **2**, 38–47.
- Kabsch, W. (2010a). *Acta Cryst.* **D66**, 125–132.
- Kabsch, W. (2010b). *Acta Cryst.* **D66**, 133–144.
- Karlin, S. & Altschul, S. F. (1990). *Proc. Natl Acad. Sci. USA*, **87**, 2264–2268.
- Lebedev, A. A. & Isupov, M. N. (2014). *Acta Cryst.* **D70**, 2430–2443.
- Long, F., Vagin, A. A., Young, P. & Murshudov, G. N. (2008). *Acta Cryst.* **D64**, 125–132.
- Maris, C., Dominguez, C. & Allain, F. H.-T. (2005). *FEBS J.* **272**, 2118–2131.
- Murshudov, G. N., Skubák, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F. & Vagin, A. A. (2011). *Acta Cryst.* **D67**, 355–367.
- Myers, J. C. & Shamoo, Y. (2004). *J. Mol. Biol.* **342**, 743–756.
- Nishiyama, H., Higashitsuji, H., Yokoi, H., Itoh, K., Danno, S., Matsuda, T. & Fujita, J. (1997). *Gene*, **204**, 115–120.
- Sachs, A. B., Davis, R. W. & Kornberg, R. D. (1987). *Mol. Cell. Biol.* **7**, 3268–3276.
- Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: A Laboratory Manual*, 3rd ed. New York: Cold Spring Harbor Laboratory Press.
- Sheikh, M. S., Carrier, F., Papathanasiou, M. A., Hollander, M. C., Zhan, Q., Yu, K. & Fornace, A. J. (1997). *J. Biol. Chem.* **272**, 26720–26726.
- Swanson, M. S., Nakagawa, T. Y., LeVan, K. & Dreyfuss, G. (1987). *Mol. Cell. Biol.* **7**, 1731–1739.
- Vagin, A. A. (1989). *Jnt CCP4/ESF-EACBM Newsl. Protein Crystallogr.* **24**, 117–121. <http://www.ccp4.ac.uk/newsletters/No24.pdf>.
- Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* **D66**, 22–25.
- Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.
- Yang, C. & Carrier, F. (2001). *J. Biol. Chem.* **276**, 47277–47284.
- Yang, R., Weber, D. J. & Carrier, F. (2006). *Nucleic Acids Res.* **34**, 1224–1236.
- Yang, R., Zhan, M., Nalabothula, N. R., Yang, Q., Indig, F. E. & Carrier, F. (2010). *J. Biol. Chem.* **285**, 8887–8893.
- Zhang, Y., Wu, Y., Mao, P., Li, F., Han, X., Zhang, Y., Jiang, S., Chen, Y., Huang, J., Liu, D., Zhao, Y., Ma, W. & Songyang, Z. (2016). *Nucleic Acids Res.* **44**, 761–775.