Cloning, expression, purification and crystallization of *Schizosaccharomyces pombe* Set7, a putative histone methyltransferase

Damiaan E. H. F. Mevius,^{a,b} Yunpeng Shen,^{a,b} Masayo Morishita^a and Eric di Luccio^{a*}

Received 10 December 2015

Accepted 6 March 2016

Edited by A. Nakagawa, Osaka University, Japan

Keywords: histone modifications; histone methyltransferase; *Schizosaccharomyces pombe*; Set7; X-ray crystallography.

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

^aGenetic Engineering Department, School of Life Sciences, College of Natural Sciences, Kyungpook National University, 80 Daehak-ro, Buk-gu, Daegu 41566, Republic of Korea, and ^bDepartment of Food Biomaterials, College of Agriculture and Life Sciences, Kyungpook National University, 80 Daehak-ro, Buk-gu, Daegu 41566, Republic of Korea.
*Correspondence e-mail: eric.diluccio@gmail.com

Dysfunction of histone-modifying enzymes affects chromatin regulation and is involved in carcinogenesis, tumour progression and other diseases. Histone methyltransferases are a family of key histone-modifying enzymes, but their structures, functions and mechanisms are incompletely understood, thus constraining drug-design efforts. Here, preliminary steps towards structure–function studies of *Schizosaccharomyces pombe* Set7, a putative histone methyltransferase and the first yeast full-length SET-domain-containing protein to be studied using X-ray crystallography, are reported. The methods from cloning to X-ray diffraction and phasing are discussed and the results will aid in prospective studies of histone-modifying enzymes.

1. Introduction

The intricate dynamics of euchromatin and heterochromatin maintenance regulate gene transcription. Large families of enzymes that modify, read and erase epigenetic marks on both DNA and histones are orchestrated in a molecular ballet, the mechanisms of which are not yet clearly understood. Histones are modified through phosphorylation, methylation, acetylation, ubiquitylation, citrullination and SUMOylation, amongst others. Subsequently, epigenetic readers recognize these specific marks, which may lead to the recruitment of various components of the transcription complex to either initiate or repress transcriptional programs. The tightly regulated interplay surrounding histone marks and chromatin states can be considered to be a ‘histone code’ that has yet to be deciphered (Zhang *et al.*, 2012; Strahl & Allis, 2000). Abnormality in the regulation of either DNA-modifying or histone-modifying enzymes is associated with an increasing number of pathologies, especially carcinogenesis and tumour progression (Cohen *et al.*, 2011; Varier & Timmers, 2011). Therefore, a better understanding of chromatin maintenance, remodelling and regulation by epigenetic modifying enzymes is of paramount importance.

Histone methylation on lysine and arginine residues mediated by the catalytic SET [Su(var)3–9, enhancer of zeste, trithorax] domain of histone methyltransferase (HMTase) is a key modification in chromatin maintenance. The SET domain of HMTase transfers one, two or three methyl groups from the

coenzyme *S*-adenosylmethionine onto lysine or arginine residues, which can have both repressive and activating effects on transcription. The SET domain is conserved across species. Genomic and proteomic studies have identified at least 77 SET-domain-containing proteins in the human proteome, whereas in the fission yeast *Schizosaccharomyces pombe* 13 SET-containing proteins have been identified (Set1–Set3, Clr4 and Set5–Set13), which are encoded by the following genes: *set1*⁺ (SPCC306.04c), *set2*⁺ (SPAC29B12.02c), *set3*⁺ (SPAC22E12.11c), *clr4*⁺ (SPBC428.08c), *set5*⁺ (SPCC1739.05), *set6*⁺ (SPBP8B7.07c), *set7*⁺ (SPCC297.04c), *set8*⁺ (SPAC3C7.09), *set9*⁺ (SPCC4B3.12), *set10*⁺ (SPBC1709.13c), *set11*⁺ (SPCC1223.04c), *set12*⁺ (SPBC543.11c) and *set13*⁺ (SPAC688.14) (Wu *et al.*, 2010; Herz *et al.*, 2013).

Amongst the 13 *S. pombe* SET enzymes, Set1, Set2, Clr4, Set9, Set10, Set11 and Set13 have been reported to possess methyltransferase activity (Noma & Grewal, 2002; Morris *et al.*, 2005; Sadaie *et al.*, 2008; Shirai *et al.*, 2010).

Set1, the homologue of the mammalian MLL H3K4 methyltransferase, is a component of the COMPASS complex that is responsible for the monomethylation, dimethylation and trimethylation of H3K4 (Noma & Grewal, 2002). Set1 is involved in the ATM kinase Rad3-dependent pathway and is responsible for telomere maintenance and DNA repair (Kanoh *et al.*, 2003). Set2 methylates H3K36 and is involved in transcriptional elongation (Morris *et al.*, 2005). Clr4, the homologue of the mammalian Suv39h1 H3K9 methyltransferase, is the sole H3K9 methyltransferase in *S. pombe* (Nakayama *et al.*, 2001). Set9 is the sole H4K20 methyltransferase responsible for the recruitment of the checkpoint protein Crb2 during DNA-damage responses (Sanders *et al.*, 2004). Set11 methylates ribosomal protein L12 (Rpl12) during ribosomal assembly (Sadaie *et al.*, 2008) and Set13 has been found to monomethylate Rpl42/43, which is associated with cell proliferation and ribosomal function (Shirai *et al.*, 2010).

Structural studies of histone-modifying enzymes are lagging behind other molecular and cellular biology studies. Of the 77 SET-containing enzymes identified in *Homo sapiens*, 34 lysine-HMTase structures, including only four full-length lysine-HMTases (SMYD2, SMYD3, SETD6 and SETD7), have been solved. In addition, out of 13 arginine-HMTase structures, ten full-length arginine-HMTases (PRMT5, PCMT1, FBL, METTL21A, METTL21B, METTL21C, METTL21D, CAMKMT, PRMT1, PRMT6 and CARM1) have been solved. Only two partial *Saccharomyces cerevisiae* HMTase structures have been solved: the N-terminal RRM domain of Set1 (PDB entry 2j8a; L. Tresaugues *et al.*, unpublished work) and the Set2 SRI domain (PDB entry 2c5z; Vojnic *et al.*, 2006). Little is known about the structural details of the *S. pombe* SET-containing proteins except for the crystal structure of the Clr4 catalytic SET domain (Min *et al.*, 2002).

In this study, in order to better understand histone methylation, we focused on the full-length *S. pombe* Set7, which contains a predicted structurally conserved SET domain. Little is known about Set7, especially its structure, enzymatic activity, substrate specificity and the characterization of Set7 mutants. Here, we crystallized Set7 from *S. pombe* with the

Table 1

Macromolecule-production information.

Source organism	<i>S. pombe</i>
DNA source	<i>S. pombe</i> meiotic cDNA library
Forward primer†	5'-GATACTAGTATGCGGATTCCTGTTATTCG-3'
Reverse primer‡	5'-GAACCTCGAGTAACTAATATTTTGAAGGG-3'
Cloning vector	pTYB12-HA (custom-modified plasmid with an HA tag on the C-terminus of the recombinant protein)
Expression vector	pTYB12-HA
Expression host	<i>E. coli</i> BL21 (DE3)
Complete amino-acid sequence of the construct produced	AGHMTSMRIPVIRSPLEIRDTERKGRGVFALEPI-PAQTCIEISPVLMFSKEEYEQHGQYTVLNEYTYVWSEGGKGLALGLGSMFNHRHPNVYWKDN-RNNYISYYTLREIKTNEELCISYGDHLWFEDASSASRISPNNEEDFPLQNISLLETMYPYDV-PDYAAPG

† The SpeI site is underlined. ‡ The XhoI site is underlined.

aim of obtaining the first crystal structure of a full-length yeast SET-domain-containing methyltransferase and thus contributing to a better understanding of the structure–function relationship of HMTs.

2. Materials and methods

2.1. Macromolecule production

2.1.1. Cloning. Full-length *set7*⁺ (SPCC297.04c, 444 bp, 147 amino acids) was amplified using an *S. pombe* meiotic cDNA library (Table 1). The *set7*⁺ PCR product flanked with SpeI and XhoI was inserted into the MCS of the protein-expression intein-tag vector pTYB12-HA, which was modified by inserting an HA tag (TMYPYDVPDYAA) at the XhoI and SmaI sites in the MCS of pTYB12 (New England Biolabs, Hitchin, England). The resultant pTYB12-*set7*⁺-HA plasmid produces the intein-Set7 fusion protein with an HA tag at its C-terminus and several extra amino acids from the remaining restriction sites (Table 1). The construct was verified by sequencing (Solgent, Daejeon, Republic of Korea).

2.1.2. Protein expression and purification. *Escherichia coli* BL21 (DE3) cells transformed with the pTYB12-*set7*⁺-HA plasmid were cultured in LB medium containing 100 mg l⁻¹ ampicillin and Set7 protein expression was induced with 125 µM isopropyl β-D-1-thiogalactopyranoside for 16 h at 12°C. Cells were harvested and lysed for 60 min in buffer A (20 mM Tris pH 8.5, 500 mM NaCl, 0.1 mM EDTA) containing 0.1% Triton X-100 and 1 mM phenylmethanesulfonyl fluoride followed by 20 cycles of sonication (2.5 min at amplitude 80) on ice. Cell debris was removed by centrifugation for 30 min at 12 000g at 4°C. The lysate was transferred to a new bottle and spun down for 30 min at 12 000g at 4°C and filtered using Corning 250 ml vacuum filters (Corning, New York, USA). The lysate containing CBD (chitin-binding domain)-intein-Set7 fusion protein was loaded onto a 50 ml column containing 15 ml chitin beads (New England Biolabs). To remove impurities, the column was washed with 100 bed volumes of buffer A containing 0.1% Triton X-100 followed by 20 bed volumes of buffer A without Triton X-100. The bacterial chaperone was removed by washing with seven bed volumes of buffer A

containing 10 mM ATP and 2.5 mM MgCl₂ followed by washing with 20 bed volumes of buffer A. Cleavage of the intein tag was induced by incubation in buffer A with 50 mM β -mercaptoethanol at 4°C for 64 h. *S. pombe* Set7 protein (19.57 kDa) was eluted in 65 ml buffer A, concentrated and washed with buffer A using 10K Amicon Ultra centrifugal filters (EMD Millipore, Billerica, Massachusetts, USA).

A secondary gel-filtration purification step was performed using an ÄKTAprime plus chromatography system with a Superdex 75 10/300 column (GE Healthcare, Little Chalfont,

Table 2
Crystallization.

Method	Hanging drop
Plate type	VDX 24-well plates
Temperature (K)	286
Protein concentration (mg ml ⁻¹)	6
Buffer composition of protein solution	10 mM Tris buffer pH 7.5
Composition of reservoir solution	0.2 M sodium phosphate dibasic, 20% (w/v) PEG 3350 pH 8.0
Volume and ratio of drop	2.4 μ l total volume: 1 μ l protein solution, 1 μ l reservoir buffer, 0.4 μ l additive (0.1 M urea stock, final concentration 16.67 mM)
Volume of reservoir (μ l)	500

Figure 1

Expression and purification of Set7. (a) Before gel filtration. Lane SDS, Coomassie Blue staining of 15% SDS-PAGE gel loaded with 5 μ g Set7; lane WB, identification of Set7 by Western blot. Set7 was detected at the expected size of 19.57 kDa using HRP-conjugated α -HA antibody. The positions of molecular-weight markers are labelled in kDa on the left. (b) After gel filtration. Lane SDS, Coomassie Blue staining of 10% SDS-PAGE gel loaded with 5 μ g Set7; lane WB, identification of Set7 by Western blot. Set7 was detected at the expected size of 19.57 kDa using HRP-conjugated α -HA antibody. The positions of molecular-weight markers are labelled in kDa on the left. (c) Size-exclusion chromatography elution profile of Set7 purification. Fractions 9–17 containing purified Set7 were pooled, concentrated and used for crystallization.

England) in buffer A with a flow rate of 0.4 ml min⁻¹. Owing to pressure limitation on the ÄKTAprime plus using the Superdex 75 10/300 column, the flow rate is below the minimum recommended flow rate of 0.5 ml min⁻¹, which might explain the broad elution-peak profile (Fig. 1). Fractions were pooled and the buffer was switched to 10 mM Tris pH 7.5 using 10K Amicon Ultra centrifugal filters. The protein was concentrated to 6 mg ml⁻¹ and confirmed by 15% SDS-PAGE stained with Coomassie Blue G-250 and Western blotting using HRP-conjugated α -HA antibody (A190-108P, Bethyl Laboratories, Montgomery, Texas, USA). SDS-PAGE and native PAGE tend to indicate homogenous *S. pombe* Set7 during the crystallization phases (Fig. 2b).

2.2. Crystallization

S. pombe Set7 protein (19.57 kDa) was crystallized using the hanging-drop method in 24-well plates (Hampton Research, Aliso Viejo, California, USA). Drops were set up using 1 μ l of 6 mg ml⁻¹ Set7 protein in 10 mM Tris buffer pH 7.5 with 1 μ l of reservoir buffer followed by the addition of 0.4 μ l of 0.1 M urea as an additive (Additive Screen, Hampton Research). Cover slides were sealed with type B mineral oil (Cargille Laboratories, Cedar Grove, New Jersey, USA) and incubated at 13°C. Crystals developed in two separate conditions: 0.2 M sodium phosphate dibasic, 20% (w/v) PEG 3350 pH 8.0 and 0.2 M magnesium formate dihydrate, 20% (w/v) PEG 3350 pH 7. In both conditions, Set7 quasi-crystals formed within 3 d that developed into solid crystals within 50 d (Fig. 2a). Crystals were resolved on SDS-PAGE to ensure the quality and the molecular weight of 19.57 kDa. The crystals were dyed with Izit dye (Hampton Research) to distinguish macromolecular crystals from those of inorganic or small molecules. Birefringence of the macromolecular crystals was assessed under polarized light using a Nikon SMZ18 microscope. Crystallization information is summarized in Table 2.

2.3. Data collection, processing, reduction and phasing

2.3.1. Data collection and processing. Diffraction data were collected on the Structural Biology Beamline 7A at the Pohang Accelerator Laboratory (PAL), Pohang University of Science and Technology, Republic of Korea. Crystals were harvested at the beamline and the best diffracting crystal was

found in condition *A* [0.2 *M* sodium phosphate dibasic, 20% (w/v) PEG 3350 pH 8.0]. Optimum diffraction was observed without cryoprotectant. However, light ice-ring formation was observed and removed by transferring the crystals to condition *B* [0.2 *M* magnesium formate dihydrate, 20% (w/v) PEG 3350 pH 7] for 30–90 s, after which the crystals were harvested and mounted under a cryostream (100 K). *S. pombe* Set7 could also be crystallized using condition *B* but gave crystals of lower quality. Since no ice ring was observed in condition *B* without cryoprotectant and condition *A* yielded the best diffracting crystals, these were briefly washed in condition *B*. Eventually, a single data set (180 images at 1° oscillation) was collected at a wavelength of 0.97930 Å. The crystal size used in this study was $\sim 75 \times 25 \times 50 \mu\text{m}$.

2.3.2. Data reduction and phasing. The crystal degraded quickly on the beamline after 66 images (1° oscillation); thus, the first 66 images of 180 were selected for further data reduction. The data were reduced using *HKL-2000* to 2.45 Å resolution in space groups *P*₆₅22 and *P*₆₁22. There are two molecules in the asymmetric unit, and molecular replacement using the crystal structure of the *Plasmodium falciparum* putative histone methyltransferase PFL0690c (PDB entry 4rz0; Structural Genomics Consortium, unpublished work) with a sequence identity of 36% was used to phase the data.

Table 3

Data collection and processing.

Diffraction source	Beamline 7A, PAL
Wavelength (Å)	0.97933
Temperature (K)	100
Detector	ADSC Q270
Crystal-to-detector distance (mm)	280
Rotation range per image (°)	1
Total rotation range (°)	66
Exposure time per image (s)	5
Space group	<i>P</i> ₆ ₁ 22 or its enantiomorph <i>P</i> ₆ ₅ 22
<i>a</i> , <i>b</i> , <i>c</i> (Å)	66.75, 66.75, 255.92
α , β , γ (°)	90, 90, 120
Mosaicity (°)	0.782
Resolution range (Å)	50.000–2.448
Total No. of reflections	90616
No. of unique reflections	12458
Completeness (%)	92.9 (99.1)
Multiplicity	7.3 (7.7)
$\langle I/\sigma(I) \rangle$	24.1 (1.74)
$R_{\text{r.i.m.}}$	0.074 (0.469)
Overall <i>B</i> factor from Wilson plot (Å ²)	55.5

Chain *A* of PDB entry 4rz0 was used as a search object for two *S. pombe* Set7 molecules in the asymmetric unit. The best molecular-replacement solution was found with a translation-function *Z*-score (TFZ) of 5.2. *Coot*, *REFMAC5* and *BUSTER* were used to build and refine the structure (Emsley *et al.*, 2010;

Figure 2

Progression of crystallization phases. (a) Crystal formation of Set7 passed through various stages. Small quasi-crystals appeared after 3 d and developed into larger quasi-crystals by day 7. The drop developed into a phase separation, which dissolved after two weeks, producing solid single crystals by around day 50. (b) SDS-PAGE and native PAGE analysis of crystallization phases. Day 0 indicates purified protein prior to crystallization. The positions of molecular-weight markers are labelled in kDa on the left.

Murshudov *et al.*, 2011; Smart *et al.*, 2012; Otwinowski & Minor, 1997; Table 3).

3. Results and discussion

Crystal structures of HMTases are needed to better comprehend chromatin dynamics and diseases caused by dysfunction of chromatin remodellers. Therefore, the rationale of this study was to shed light on full-length structures of HMTs to contribute to elucidating chromatin maintenance and regulation along with associated diseases. In this study, we cloned, expressed and crystallized an *S. pombe* putative HMTase, Set7 which is the first full-length yeast HMTase structure to be solved.

An *S. pombe* meiotic cDNA library was used to clone *set7*⁺ into a modified pTYB12-HA plasmid fused with an intein tag at the N-terminus and an HA tag at the C-terminus of *set7*⁺. Noteworthy, out of 13 *S. pombe* SET-containing genes, only *set6*⁺ and *set7*⁺ contain introns. The HA tag (TMYPYDVP-DYAA) was added to increase the stability and solubility of the recombinant protein, while allowing protein identification by immunoblot. Approximately 15 g of cells from a 9 l LB protein-expression batch were lysed and Set7 protein was purified using the intein-tag affinity column, yielding 1.1 mg of purified protein. The protein was further purified by gel filtration using a Superdex 75 10/300 column (Fig. 1).

For crystallization of Set7, the protein concentration was increased to 12 mg ml⁻¹ in 10 mM Tris buffer pH 7.5. Initial

hits showing quasi-crystals or small crystals were reproduced at a lower concentration of 6 mg ml⁻¹. Solid useful crystals appeared within 50 d of incubation at 13°C and transitions through multiple states are shown in Fig. 2. Microcrystals appeared within 72 h, which next developed into quasi-crystals by day 7 and part of the drop further evolved into a phase-separation state. Solid crystals appeared within 21 d following the disappearance of the phase-separation state (Fig. 2a). The dynamic process of protein phase change was investigated by SDS-PAGE and native PAGE, which may suggest that *S. pombe* Set7 remains in a homogenous state during the crystallization phase (Fig. 2b). However, some degradation of *S. pombe* Set7 was observed (Fig. 2b).

To investigate the authenticity of the crystals, two additional control experiments were performed. The blue IZIT dye was readily absorbed in protein crystal solvent channels, resulting in dark blue crystals, clearly supporting that the crystals were macromolecular and not of inorganic salts. Secondly, crystals were examined under polarized light and clearly showed birefringence (Supplementary Fig. S1).

The crystals were found to be delicate and difficult to handle. Manual harvesting of the crystals at the synchrotron was found to be the best way to minimize stress and ice-ring formation and to maximize diffraction quality. The best diffraction, to 2.45 Å resolution, was observed with crystals incubated in 0.2 M sodium phosphate dibasic, 20% (w/v) PEG 3350 pH 8.0 with no additional cryoprotectant (Supplementary Fig. S2). As the space group was uncertain, 180 diffraction images at 1° oscillation were collected with a 5 s exposure time. However, the crystal quality deteriorated during data collection owing to radiation damage (data not shown). Data reduction was performed using the first 66 images, and the crystal unit-cell parameters were identified as $a = b = 66.8$, $c = 255.9$ Å. The space group is ambiguous, being either $P6_122$ or its enantiomorph $P6_522$, both with $\alpha = \beta = 90$, $\gamma = 120^\circ$. Cell-content analysis indicated the likelihood of two monomers per asymmetric unit, with a solvent content of 49.35% and a V_M of 2.43 Å³ Da⁻¹ (Table 3).

Only a native data set could be collected; therefore, molecular replacement was used to phase the data. An NCBI BLAST search against the Protein Data Bank for the *S. pombe* Set7 sequence returned the crystal structure of *P. falciparum* putative histone methyltransferase PFL0690c (PDB entry 4rz0) with 36% sequence identity as a possible candidate for molecular replacement. As the space group is ambiguous, using *Phaser* for molecular replacement we explored the enantiomorphic $P6_122$ and $P6_522$ space groups with PDB entry 4rz0 as a monomeric search model. A potential solution was found in space group $P6_522$ with two molecules in the asymmetric unit. Initial electron-density map inspection showed unambiguous and continuous density around the search model. After several rounds of model rebuilding with *Coot* and refinement with both *REFMAC5* and *BUSTER*, the electron-density maps became convincing, with clear continuous $2F_o - F_c$ electron density contoured at 1.5σ and positive $F_o - F_c$ electron density contoured at 3σ (Winn *et al.*, 2011; McCoy *et al.*, 2007; Vagin *et al.*, 2004; Smart

Figure 3
Electron-density maps. $2F_o - F_c$ electron-density maps are contoured at 1.0σ (blue) and $F_o - F_c$ electron-density maps are contoured at 3.0σ (green, positive; red, negative). (a) Electron densities in *Coot* are displayed side-by-side with $2F_o - F_c$ OMIT and $F_o - F_c$ OMIT maps for residues 6–15 (b).

et al., 2012; Fig. 3). The identity of these two proteins is relatively low: the search model PDB entry 4rz0 is weakly related to *S. pombe* Set7, with only 36% sequence identity. Therefore, owing to backbone-tracing errors, the *R* factor and R_{free} are currently at 43.8 and 45.4%, respectively. It is noteworthy that 96 residues out of 334 (28.7%) are still missing at this early stage of model building and refinement. Specifically, the missing amino acids are as follows. In chain *A*, amino acids –6 to 0, residues added owing to cloning at the N-terminus; amino acids 58–74, residues predicted to be a conserved α -helix; amino acids 106–115, residues predicted to be a flexible loop; and amino acids 147–163, an HA tag added at the C-terminus for increased protein stability. In chain *B*, amino acids –6 to 0, residues added owing to cloning at the N-terminus; amino acids 58–71, residues predicted to be a conserved α -helix; amino acids 106–115, residues predicted to be a flexible loop; and amino acids 147–163, an HA tag at the C-terminus. Protein-backbone as well as side-chain placement is clearly visible with convincing electron-density maps (Fig. 3). The initial analysis of the structure does not support a biological dimer in the crystal, consistent with gel electrophoresis of the purified soluble protein. In order to ensure the validity of the molecular-replacement solution and subsequent model-building steps, $2F_o - F_c$ and $F_o - F_c$ electron-density OMIT maps were calculated (Fig. 3*b*). Residues between Ile6 and Thr15 were omitted and both the $2F_o - F_c$ OMIT map contoured at 1σ and the $F_o - F_c$ OMIT positive electron density contoured at 3σ support a valid phasing solution.

Taken together, the details of the crystallization process and the phasing of *S. pombe* Set7 will contribute to the structural biology studies of HMTases in eukaryotes.

Acknowledgements

The *S. pombe* meiotic cDNA library was a gift from Professor Taro Nakamura, OCU, Japan. This study is supported in part by research grants from Astex Pharmaceuticals, Cambridge, England (KNU 2014-04260000 and KNU 2014-1440000) and the Basic Science Research Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2013R1A1A-2012486). The Kyungpook National University Research Fund also contributed to the research presented in this study. We also acknowledge the staff at Pohang Accelerator

Laboratory, Pohang University of Science and Technology, Republic of Korea.

References

- Cohen, I., Poręba, E., Kamieniarz, K. & Schneider, R. (2011). *Genes Cancer*, **2**, 631–647.
- Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.
- Herz, H. M., Garruss, A. & Shilatfard, A. (2013). *Trends Biochem. Sci.* **38**, 621–639.
- Kanoh, J., Francesconi, S., Collura, A., Schramke, V., Ishikawa, F., Baldacci, G. & Géli, V. (2003). *J. Mol. Biol.* **326**, 1081–1094.
- McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
- Min, J., Zhang, X., Cheng, X., Grewal, S. I. S. & Xu, R.-M. (2002). *Nature Struct. Biol.* **9**, 828–832.
- Morris, S. A., Shibata, Y., Noma, K., Tsukamoto, Y., Warren, E., Temple, B., Grewal, S. I. S. & Strahl, B. D. (2005). *Eukaryot. Cell*, **4**, 1446–1454.
- 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.
- Nakayama, J., Rice, J. C., Strahl, B. D., Allis, C. D. & Grewal, S. I. S. (2001). *Science*, **292**, 110–113.
- Noma, K. & Grewal, S. I. S. (2002). *Proc. Natl Acad. Sci. USA*, **99**, 16438–16445.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Sadaie, M., Shinmyozu, K. & Nakayama, J. (2008). *J. Biol. Chem.* **283**, 7185–7195.
- Sanders, S. L., Portoso, M., Mata, J., Bähler, J., Allshire, R. C. & Kouzarides, T. (2004). *Cell*, **119**, 603–614.
- Shirai, A., Sadaie, M., Shinmyozu, K. & Nakayama, J. (2010). *J. Biol. Chem.* **285**, 22448–22460.
- Smart, O. S., Womack, T. O., Flensburg, C., Keller, P., Paciorek, W., Sharff, A., Vornrhein, C. & Bricogne, G. (2012). *Acta Cryst.* **D68**, 368–380.
- Strahl, B. D. & Allis, C. D. (2000). *Nature (London)*, **403**, 41–45.
- Vagin, A. A., Steiner, R. A., Lebedev, A. A., Potterton, L., McNicholas, S., Long, F. & Murshudov, G. N. (2004). *Acta Cryst.* **D60**, 2184–2195.
- Varier, R. A. & Timmers, H. T. (2011). *Biochim. Biophys. Acta*, **1815**, 75–89.
- Vojnic, E., Simon, B., Strahl, B. D., Sattler, M. & Cramer, P. (2006). *J. Biol. Chem.* **281**, 13–15.
- Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.
- Wu, S., Wang, W., Kong, X., Congdon, L. M., Yokomori, K., Kirschner, M. W. & Rice, J. C. (2010). *Genes Dev.* **24**, 2531–2542.
- Zhang, X., Wen, H. & Shi, X. (2012). *Acta Biochim. Biophys. Sin.* **44**, 14–27.