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Multiple polymer architectures of human Polyhomeotic homolog 3 (PHC3) SAM

David R. Nanyes, Sarah E. Junco, Alexander B. Taylor, Angela K. Robinson, Nicolle L. Patterson, Ambika Shivarajpur, Jonathan Halloran, Seth M. Hale, Yogeeet Kaur, P. John Hart¹, and Chongwoo A. Kim

The University of Texas Health Science Center San Antonio, Department of Biochemistry, ¹X-ray Crystallography Core Laboratory, MSC 7760, 7703 Floyd Curl Dr., San Antonio, TX 78229

¹Department of Veterans Affairs, South Texas Veterans Health Care System, San Antonio, TX 78229

Abstract

The self-association of sterile alpha motifs (SAMs) into a helical polymer architecture is a critical functional component of many different and diverse array of proteins. For the *Drosophila* Polycomb group (PcG) protein Polyhomeotic (Ph), its SAM polymerization serves as the structural foundation to cluster multiple PcG complexes, helping to maintain a silenced chromatin state. Ph SAM shares 64% sequence identity with its human ortholog, PHC3 SAM, and both SAMs polymerize. However, in the context of their larger protein regions, PHC3 SAM forms longer polymers compared to Ph SAM. Motivated to establish the precise structural basis for the differences, if any, between Ph and PHC3 SAM, we determined the crystal structure of the PHC3 SAM polymer. PHC3 SAM utilizes the same SAM-SAM interaction as the Ph SAM six-fold repeat polymer. Yet, PHC3 SAM polymerizes utilizing just five SAMs per turn of the helical polymer rather than the typical six per turn observed for all SAM polymers reported to date. Structural analysis suggested that malleability of the PHC3 SAM would allow formation of not just the five-fold repeat structure but possibly others. Indeed, a second PHC3 SAM polymer in a different crystal form forms a six-fold repeat polymer. These results suggest that the polymers formed by PHC3 SAM, and likely others, are quite dynamic. The functional consequence of the variable PHC3 SAM polymers may be to create different chromatin architectures.

Keywords

SAM; Polyhomeotic; Polycomb group; Chromatin; Polymer

Introduction

The Sterile Alpha Motif (SAM) is an approximately 70 residue, independently folded protein domain found in a diverse range of proteins ¹. SAMs, in general, serve as an interaction module able to self-associate, bind other proteins and even bind nucleic acids.

What has been frequently observed for self-associating SAMs is the formation of an open-ended polymer structure mediated through a series of head-to-tail interactions between two different binding surfaces on the SAMs called the mid-loop (ML) and end-helix (EH) binding surfaces (Fig. 1A). While variations exist amongst the SAM polymer structures determined to date, the utilization of these two binding surfaces and formation of the helical polymer structure utilizing six SAMs to complete one turn of the helix has been a consistent theme observed for a wide-variety of SAMs (e.g., TEL², Polyhomeotic (Ph)³, Yan⁴, Scm⁵, Shank3⁶, DGKd1⁷, Sla1p⁸, and Caskin1⁹).

Polyhomeotic (Ph) is a SAM containing *Drosophila* protein belonging to the Polycomb group (PcG) of epigenetic regulators. Ph and its human orthologs, PHC1, 2, and 3, possess growth suppressive ability¹⁰⁻¹³. All Polyhomeotic proteins contain a C-terminal SAM (Fig. 1B) whose polymerization is critical for the gene silencing function of the protein and its ability to suppress growth¹⁴. Recent studies suggest that SAM polymerization may serve as the structural foundation for the clustering of multi-protein chromatin associated complexes¹⁵ (Francis, N.J., personal communication). Ph SAM polymer assembles into a left-handed, helical architecture with six-fold screw axis symmetry³ like all other polymeric SAMs reported to date^{2, 4-9}. Like Ph, PHC3 SAM can also polymerize¹⁶. Given the 64% sequence identity shared between Ph and PHC3 SAMs (Table I), and that all the residues that mediate the Ph SAM-Ph SAM interaction are completely conserved in PHC3 SAM (Fig. 1C), it would be more than reasonable to expect PHC3 SAM to form a similar polymer architecture as Ph SAM. There are, however, significant differences in the polymerization behavior between the two proteins. Studies of Ph and PHC3 SAM polymerization revealed the key role of the intrinsically disordered stretch of residues adjacent to the SAMs in determining the length of SAM polymers that can form¹⁶. While the unstructured residues N-terminal to Ph SAM allow only short polymers to form, the corresponding residues N-terminus to PHC3 SAM allow open-ended polymerization.

To provide the precise structural framework for these differences, we determined the X-ray crystal structure of PHC3 SAM. PHC3 SAM forms a polymer that is unique amongst all previously reported SAM polymer structures. Rather than having six SAMs per turn of the SAM polymer helix, PHC3 SAM utilizes only five. The SAM-SAM interaction that mediates the PHC3 SAM five-fold polymer is identical to the one mediating the six-fold Ph SAM polymer. This suggested that the PHC3 SAM may also be able to form the six-fold repeat structure. Indeed, we found this to be the case for a PHC3 SAM crystallized in an alternative crystal form. These results suggest that SAM polymers can be dynamic, taking on alternative polymeric states. The functional consequence of this variability may be to serve as the foundation for creating different repressive chromatin states.

Material and Methods

Protein preparation

The gene encoding PHC3 SAM (914 - 983) was cloned into a modified pET-3c with a MEKTR leader sequence and RHHHHHH at the C-terminus. All mutations were introduced using QuickChange (Agilent) mutagenesis kit. Proteins were expressed in BL21(DE3) pLysS cells and purified as previously described³. Briefly, proteins were purified using

Ni²⁺ affinity chromatography followed by ion exchange chromatography. Proteins were prepared in 10 mM Tris pH 8.0, 50 mM NaCl, 1 mM TCEP.

Crystal structure determination of the five-fold repeat PHC3 SAM polymer

The five-fold repeat crystals were grown using a protein sample containing an equimolar mix of the PHC3 SAM EH mutant L971E and the ML double mutant L953R/H958R. This dimer was prepared to a total protein concentration of 51 mg/ml. Crystals were grown via vapor diffusion in 100 mM Tris pH 7.5, 55% ethylene glycol. Data were collected at the ALS beamline 4.2.2 and processed with XDS¹⁷. The space group was P1. The structure was determined using the molecular replacement method implemented in PHASER¹⁸ with Ph SAM (PDB ID 1KW4) as the search model. Iterative cycles of model building and refinement were carried out in Coot¹⁹ and PHENIX²⁰, respectively. The electron density maps with side chains omitted at the sites of mutation showed density most consistent with the L971E mutation for all SAMs in the asymmetric unit. Moreover, refinement with the L953R/H958R mutations resulted in increasing the R-factors. We therefore conclude that only the PHC3 SAM L971E mutant is in the crystal. All structure figures were made with Chimera²¹. The structure determination statistics are in Table II. The PDB ID for the PHC3 SAM L971E five-fold repeat polymer is 4PZN.

Crystal structure determination of the six-fold repeat PHC3 SAM polymer

The PHC3 SAM L967R mutant was prepared to 22 mg/ml. Crystals were grown using the hanging drop vapor diffusion method mixing equal volumes of 1.0 - 1.2 M sodium acetate (pH 5.5 - 6.0) with the protein solution. Data were collected at the UTHSCSA X-ray Crystallography Core Laboratory on a Rigaku MicroMax 007-HF rotating anode generator equipped with RAXIS-HTC image plates and X-Stream cryogenic systems and processed using XDS¹⁷. The morphology of the crystal unit cell is close to a hexagonal cell setting but is a twinned C2 system with unit cell axes $a = 123.9 \text{ \AA}$, $b = 51.9 \text{ \AA}$, $c = 124.0 \text{ \AA}$, $\beta = 119.7^\circ$ and twin operator $h, -k, -h-l$. Significant pseudo translational symmetry was also present in the data according to Patterson Function analysis. Given the combination of crystal pathologies, the space group was verified using the program Zanuda²². This six-fold screw axis arrangement of the PHC3 SAM polymer was initially suggested by a Patterson self-rotation analysis indicating a significant non-crystallographic six-fold axis parallel to the crystallographic two-fold axis of the C2 unit cell. The structure was determined using molecular replacement with a single PHC3 SAM from the five-fold repeat structure as the search model. Model building and refinement were carried out as described above. The refinement R-factors remained high, perhaps impacted by the combination of pseudo translational symmetry, twinning, and a high solvent content (60%). The data produced an electron density map of lower quality than generally expected for the resolution range; for example, the residue range 960-968 exhibited discontinuities and may actually contain multiple conformations that could not be modeled accurately in some of the monomers. Nevertheless, a composite simulated annealing omit map (Figure 4A) confirms the placement of each monomer as part of a repeating helix with a non-crystallographic six-fold screw axis. The PDB ID for the PHC3 SAM L967R six-fold repeat polymer is 4PZO.

Results

PHC3 SAM forms a five-fold repeat unit polymer

Our motivation to determine the structure of PHC3 SAM polymer was to identify the differences, if any, between Ph SAM and PHC3 SAM. We first attempted the crystallization strategy of introducing mutations at the SAM-SAM binding surface which hinder polymerization in solution but allows the native polymer structure to form under the high concentrations required for crystallization. This method yielded many lead crystals of PHC3 SAM likely owing to the high propensity of SAMs to assemble into the commonly observed crystallographic six-fold screw axis symmetry operation. Despite the many leads, most conditions could not be optimized to grow well-diffracting crystals (one twinned crystal structure determined using this strategy is described below). We thus utilized an alternative method aiming to isolate just two SAM units of polymer from which the dimensions of the extended polymer could be extrapolated. We mixed two different SAM mutants, each with a mutation on either the EH or ML binding surfaces. The remaining native binding surface on the opposite binding surface of each of these mutant SAMs would allow formation of just the SAM-SAM dimer. We used the ML binding surface mutant PHC3 SAM L953R/H958R which previously showed no self-association¹⁶. We combined this SAM mutant with the L971E EH surface mutant which showed a homogenous sedimentation profile consistent with a monomeric state¹⁶. Protein crystals were grown in the triclinic space group, P1. Rather than forming just the anticipated dimer unit of the PHC3 SAM polymer, a polymer of just the L971E mutant was observed (see methods). Remarkably, the SAM polymer in this crystal form is quite different than either the Ph SAM or any other previously described SAM polymer. Rather than the SAMs assembling into the six-fold repeat helical polymer, PHC3 SAM polymer has just five SAMs per turn of helix.

Identical SAM-SAM interactions mediates both the PHC3 SAM five-fold and Ph SAM six-fold repeat polymers

We wished to determine why two SAMs that share very high sequence identity with complete conservation of the residues that mediate their self-association (Fig. 1C) could form very different polymer architectures. We first compared the overall folds between Ph SAM and PHC3 SAM (Fig. 3A). The two SAMs are very similar to each other with a C_α RMSD of 0.77. This would make Ph SAM closer in structure to a different protein (PHC3 SAM) than it does to itself crystallized in a different context. That is because the C_α RMSD between two Ph SAMs from two different crystal structures^{3, 5} is 1.0 (Table III). It should be noted that there is variability in the folds of the five PHC3 SAMs in the asymmetric unit. The RMSDs between Ph SAM and the five PHC3 SAMs range from 0.77 to 1.1 (Table III). This variation reflects the flexibility of PHC3 SAM which could have significant consequences in polymer formation (see below).

In addition to the nearly identical folds, the PHC3 SAM-PHC3 SAM interface is the same as that observed for Ph SAM-Ph SAM. PHC3 SAM-PHC3 SAM binding is mediated through apolar PHC3 SAM EH surface residues Leu 967 and Leu 971 (mutated to Glu here) which pack against a hydrophobic ML surface binding pocket (Fig. 3B). In addition, the side chain amino group of the EH surface residue Lys 972 makes a key salt bridge interaction with the

carboxylate side chain of Asp 947 on the ML surface. All these interactions also occur in the Ph SAM polymer (Fig. 3B, right). We thus conclude that the alternative, five-fold repeat, PHC3 SAM polymer architecture occurs by utilizing the identical SAM-SAM interaction as the Ph SAM six-fold repeat polymer.

We wondered how two proteins with nearly identical folds and modes of self-association could form an alternative higher order assembly. To understand how this occurs, we overlaid a single Ph SAM and PHC3 SAM, then viewed how the next SAM in the polymer is arranged. As noted above, when individual SAMs are overlaid, the SAMs align very well (Fig. 3C, left SAM). This is noted by very close placement of the residues that constitute the ML binding surface. The EH surface, comprised of the residues at the start of the last helix (PHC3 966 - 971, Ph 1560 - 1565) of the SAM on the right, are also overlaid nearly identically between Ph and PHC3 SAMs. However, deviation between Ph and PHC3 SAM are very apparent more distant from the EH binding surface. These subtle changes in SAM conformation accumulated over multiple SAMs leads to formation of either the five- or six-repeat SAM polymer.

PHC3 SAM can also form a six-fold repeat helical polymer architecture

The above analysis shows that differences between Ph and PHC3 SAM folds, while very subtle for the individual SAMs, are amplified over multiple SAM-SAM interactions resulting in the formation of alternative helical polymer architectures. Like all proteins, these individual SAMs possess a certain degree of flexibility. This is indicated by the range of RMSDs between the five SAMs in the asymmetric unit of the five-fold SAM polymer crystal (Table III). Thus, rather than Ph and PHC3 SAM being completely rigid structures that only form the six or five-fold repeat structures, respectively, their malleability likely allows them to form different polymeric states. We wished to demonstrate that this is the case by showing that PHC3 SAM can also form a six-fold repeat structure. Using PHC3 SAM EH mutant L967R, we grew well-diffracting crystals different from the P1 space group that produced the five-fold repeat structure. This alternative crystal form contains a six-fold repeat PHC3 SAM polymer. This was in part confirmed through generation of a composite simulated annealing omit electron density map which shows clear density for all six PH3 SAMs in the asymmetric unit (Fig. 4A, see also methods). The dimensions of the polymer are different than either Ph or the PHC3 SAM five-fold repeat polymers (Fig. 4B). In particular, the repeat distance for PHC3 SAM six-fold repeat is 52 Å (8.7 Å per SAM) compared to 45 Å for the six-fold repeat Ph SAM polymer (7.5 Å per SAM) or even the 35 Å for the five-fold PHC3 SAM (7.0 Å per SAM). Again, overlaying one SAM and assessing how the neighboring SAM differs for each polymer shows that the malleability of SAMs allows this particular polymer to form (Fig. 4C). Together, these results show that the flexibility allows SAMs to be dynamic and capable of forming multiple polymer architectures that differ in the number of units per turn and the repeat distance of the polymer.

Discussion

We present two different polymeric architectures of PHC3 SAM. These findings suggest that SAM polymers are dynamic and not limited to a single state. The reason why a five-fold repeat SAM polymer architecture had not been observed previously is likely due to the inherent ability of SAMs to crystallize into the common crystallographic symmetry operation. Indeed, SAMs have been co-opted for their high propensity to crystallize by fusing them to crystallize other target proteins²³. There are no five-fold crystallographic symmetry operations. They do, however, appear as non-crystallographic symmetry elements, which is the case here. The crystallization of the five-fold repeat resulted from having two different SAMs during crystallization which were intended to form only a dimeric unit of the polymer. Despite this strategy, a five-fold SAM polymer of just one of the SAM mutants still forms in the crystal. It seems plausible that the role of the partnering SAM that did not crystallize was to slow down the crystallization of the six-fold repeat polymer allowing the five-fold polymer to effectively compete in crystallizing. Given this result, the abundance of six-fold polymeric SAMs found in crystal structures may be an over representation of SAM polymers that actually occur in solution.

It is important to note the caveats of this study. Mutating any residue may destabilize the PHC3 SAM fold making it more prone to crystallization in a non-native conformation. However, five-fold screw axes, which the L971E mutant crystallizes as, occur only as non-crystallographic symmetry elements while the six-fold screw axis is a common crystallographic symmetry operation. Thus, it would seem that a SAM more prone to being an artifact of crystallization would be more inclined to crystallize into the six-fold screw axis. Moreover, there are fewer crystal contacts that surround the five repeat polymer compared to the six (Supp. Fig. 1) suggesting less influence from crystal packing. Alternative to being a crystallographic artifact, it is possible that the five repeat PHC3 SAM polymer constitutes a significant portion of the polymers in solution which crystallized under a very specific set of circumstances. Ultimately, a different PHC3 SAM mutant that forms the five repeat polymer will be needed to provide further evidence for the ability of PHC3 SAM to form this novel polymer architecture.

The strategy that induced crystallization of the five-fold repeat polymer also points to a potential way in which different PHC3 SAM polymer architectures could be created in cells. While the double L953R/H958R ML surface mutant may have slowed down crystallization of the six-fold repeat of the L971E EH mutant thereby allowing the five-fold repeat structure to form, it also is possible that slight conformational changes induced upon SAM-SAM binding could favor one polymer form over the other. There are multiple binding partners to PHC SAMs which may subtly influence the conformation of the SAM which over multiple SAMs in the polymer could influence the formation of a particular polymer architecture. For example, the SAM of a PcG protein called Sex comb on midleg (SCMH1) is known to interact directly with the PHC1 SAM²⁴. The equivalent interaction is also observed for the *Drosophila* proteins^{5, 25, 26}. In addition to SCMH1 SAM, the SAMs of the three PHCs appear likely to interact with each other given the nearly 80% sequence identity between them and conservation of all residues that mediate SAM-SAM interactions (Table I). To this point, PHC1 and PHC2 do co-localize in cells¹⁵ and function in a cooperative manner²⁷.

Unfortunately, we have not yet been able to obtain SAMs of PHC1 and PHC2 to test this hypothesis. Supportive of the idea of SAM malleability is that the structure of Ph SAM from the polymer is closer in structure (based on C α RMSDs) to PHC3 SAM than the Ph SAM that is in complex with Scm SAM (Table III). Given this variation present within Ph SAM, it seems plausible that Ph SAM, like PHC3 SAM, would be able to form alternative polymeric architectures.

The malleability of PHC3 SAM which allows it to form different polymer architectures may not be attributable to all SAMs. Scm SAM has also been crystallized in two different contexts: one as a polymer and, as noted above, in complex with Ph SAM⁵. The very low C α RMSDs (0.46 ± 0.07 Å, $n = 6$) when comparing Scm SAMs in the two different crystals suggest Scm SAM may not be as malleable as PHC3 SAM (avg. C α RMSD = 0.99 ± 0.12 Å, $n = 30$ between PHC3 SAMs in the five and six repeat polymer structures). The SAM from the protein TEL has also been crystallized in multiple contexts. The C α RMSDs between TEL SAMs that form a polymer in the crystal compared to TEL SAMs that only form dimeric units of the polymer are also lower (avg. C α RMSD = 0.65 ± 0.13 Å, $n = 18$) compared to the PHC3 SAM differences. Furthermore, regardless of these different contexts, the TEL SAM-TEL SAM interaction appears invariable as the helical architectures modeled from the two different crystal structures both exhibit a six repeat polymer with nearly identical repeat distances (53 Å for the polymer², 54 Å when extrapolated from the dimer structure²⁸). While this analysis is quite limited, it does suggest that PHC3 SAM (and likely Ph SAM) has greater flexibility than either Scm or TEL SAM (see Supplementary Tables SI – SIII for all calculated C α RMSDs).

The different number of SAMs that make up a turn of the SAM polymer helix can have considerable impact on its ability to regulate chromatin structure. Recent studies suggest that the molecular role of the SAM polymer is to serve as the structural foundation for higher order repressive chromatin states¹⁵. A chromatin structure mediated by five PHC3 SAMs versus six SAMs per turn of the helical architecture would significantly dilute the protein domains that surround the SAM helical architecture and ultimately lead to different chromatin structures. While we have now observed five and six repeats per turn of a SAM polymer, there are likely to be even greater variability. It has recently been observed that the SAM of the ANKS3 protein can form a polymer with eight SAMs per repeat of the helix (Leettola, C. & Bowie, J. U., personal communication). It will be important to know if this and other SAMs are also as malleable as PHC3 SAM and if so, how many different polymer states they can form.

Conclusion

We conclude that PHC3 SAM is dynamic and can self-associate into different polymer architectures. The five-fold repeat polymer structure would present a more diluted display of protein domains that surround the SAM helical architecture compared to the six-fold repeat polymer. Consequently, these alternative SAM polymer architectures may represent different higher order chromatin structures that are created at different genomic locations or cause differential gene expression patterns.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
PHC3 SAM is very similar to Ph SAM. (A) Illustration of SAM polymerization. Each SAM has two binding surfaces called the Mid-Loop (ML) and End-Helix (EH) surfaces. The ML of one SAM binds to the EH of another which leads to polymerization. (B) Domain structure of PHC3 SAM. All Polyhomeotic share the same domain structure. HD1: homology domain 1; FCS: Phe-Cys-Ser domain. (C) Sequence alignment of Ph and all three human orthologs, PHC1, 2, and 3. Light and dark shaded residues indicate the ML and EH surfaces, respectively. These residues are completely conserved for all human PHC proteins.

Figure 2. PHC3 SAM forms a five-fold repeat polymer structure. Two different views of PHC3 and Ph SAM polymers. Top shows a ribbon representation of the SAMs viewing down the helical axis of the SAM polymers. Each SAM comprising the helical repeat is represented by a different color. Bottom is a surface representation side view of the SAM polymers. The structure of the Ph SAM polymer was previously determined³ (PDB ID 1KW4). It is shown here again to allow direct comparison with the PHC3 SAM five-fold repeat polymer. The pitch and width distances for the Ph SAM polymer are 45 Å and 70 Å, respectively.

Figure 3.

Small differences in SAM fold alters polymer architecture. (A) Overlay of Ph SAM (red, 1KW4) with the best matching PHC3 SAM (gold) of the five PHC3 SAMs in the asymmetric unit. (B) The SAM-SAM interface of PHC3 SAM and Ph SAM. The ML surface SAM is shown as the gold surface representation. The ML surface is a hydrophobic pocket mediated by the indicated residues. The EH surface is shown as a pink ribbon. Leu 967 and Leu 971 (mutated to Glu for PHC3 SAM) pack against this ML pocket. (C) Slight deviations in SAM structure allow PHC3 SAM to form the five-fold repeat polymer. Two consecutive SAMs of PHC3 (gold) and Ph (red) are shown. Alignment was performed on just the SAM on the left. The beginning of the last helix that comprises the EH surface is highlighted.

Figure 4.

PHC3 SAM can also form a six-fold repeat polymer. (A) A composite simulated annealing omit map contoured at 1σ . The electron density was contoured with a $1.8 \text{ \AA}$ radius around each atom. The C_α trace for each PHC3 SAM is shown in a different color. (B) Two views of the PHC3 SAM six-fold repeat polymer. (C) Overlay of two consecutive SAMs. The view is looking down the SAM polymer helix axis. The overlaid SAMs are on the left. PHC3 SAM six-fold repeat polymer is dark cyan in both top and bottom. Gold is PHC3 SAM from the five-fold repeat polymer, red is Ph SAM.

Table I
Sequence identity between Polyhomeotic proteins

	Ph	PHC1	PHC2
PHC1	59		
PHC2	57	79	
PHC3	64	76	80

The percentages are calculated over the alignment of 70 residues as shown in Fig. 1C.

Table II

Data collection and refinement statistics.

PDB code	1PZN	1PZO
Data collection		
X-ray source	Advanced Light Source Beamline 4.2.2	UTHSCSA X-ray Crystallography Core Laboratory
Space group	<i>P</i> 1	<i>C</i> 2
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	35.1, 60.8, 61.4	123.9, 51.7, 124.0
α , β , γ (°)	69.4, 75.9, 78.1	90, 119.7, 90
Wavelength (Å)	1.0000	1.5418
Resolution (Å)	19.68-2.30 (2.42-2.30)*	46.64-2.25 (2.37-2.25)
<i>R</i> _{sym}	0.029 (0.253)	0.062 (0.476)
<i>I</i> / σ <i>I</i>	11.4 (3.1)	9.8 (1.9)
Completeness (%)	97.8 (97.2)	99.8 (100.0)
Redundancy	2.0 (2.0)	3.5 (3.5)
Wilson value	44.5	38.9
Refinement		
Resolution (Å)	19.68-2.30	40.56-2.25
No. reflections	19,834	32,704
<i>R</i> _{work} / <i>R</i> _{free}	0.205/0.250	0.282/0.335
No. atoms		
Protein	2,706	3,321
Water	40	107
B-factors (Å ²)		
Protein	57.7	52.1
Water	51.7	43.0
R.m.s deviations		
Bond lengths (Å)	0.009	0.003
Bond angles (°)	1.237	0.596
Ramachandran outliers (%)	0.0	0.5

* Highest resolution shell is shown in parentheses.

Table III
C_α RMSDs between Polyhomeotic SAMs

	Ph (1KW4)	Ph (1PK1, chain A)	Ph (1PK1, chain C)
Ph (1PK1, chain A)	1.03		
Ph (1PK1, chain C)	1.05	0.81	
PHC3 5-fold	0.77 [*]	0.86	0.89
PHC3 6-fold	0.88 [*]	1.28	1.39

Comparisons were performed over 63 to 68 residues. The Ph SAM helical polymer PDB ID is 1KW4. The Ph SAM with PDB ID of 1PK1 is from the structure of Ph SAM in complex with Scm SAM⁵ which has two Ph SAMs in the asymmetric unit (chains A and C).

^{*} The represents the lowest RMSD between Ph SAM and the different PHC3 SAMs in the asymmetric unit of the crystal. The RMSDs range from 0.77 to 1.1 for Ph and the PHC3 SAMs of the five-fold repeat and 0.88 to 0.99 for the six-fold repeat PHC3 SAM. The PHC3 SAMs compared with 1KW4 Ph SAM were also compared with the Ph SAMs from 1PK1.