CHROMATIN-MODIFYING COMPLEX COMPONENT NURF55/P55 ASSOCIATES WITH HISTONES H3, H4 AND POLYCOMB REPRESSIVE COMPLEX 2 SUBUNIT SU(Z)12 THROUGH PARTIALLY OVERLAPPING BINDING SITES

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Drosophila Nurf55 is a component of different chromatin-modifying complexes including the Polycomb Repressive Complex 2 (PRC2). Based on the 1.75 Å crystal structure of Nurf55 bound to histone H4 helix 1, we analyzed interactions of Nurf55 (Nurf55 or p55 in fly, RbAp48/46 in human) with the N-terminal tail of histone H3, the first helix of histone H4 and an N-terminal fragment of the PRC2 subunit Su(z)12 using isothermal calorimetry and pull-down experiments. Site-directed mutagenesis identified the binding site of histone H3 at the top of the Nurf55 β-propeller. Unmodified, K9me3 or K27me3 containing H3 peptides are bound with similar affinities, while the affinity for K4me3 containing H3 peptides is reduced. Helix 1 of histone H4 and Su(z)12 bind to the edge of the β-propeller using overlapping binding sites. Our results show similarities in the recognition of histone H4 and Su(z)12 and identify Nurf55 as a versatile interactor that simultaneously contacts multiple partners.

Polycomb group (PcG) proteins play important roles in maintaining the silenced state of homeotic genes as well as in other biological processes including X-chromosome inactivation, germline development, stem cell pluripotency and cancer metastasis (reviewed in (1,2)). Thus far, three different major Polycomb Repressive Complexes (PRCs) have been identified in Drosophila melanogaster: PRC1, PRC2 and Pho-Repressive Complex (PhoRC).

Drosophila PRC1 contains four core subunits, including the chromodomain protein Polycomb (Pc) which specifically binds the trimethylated lysine 27 of the histone H3 tail. In vitro, high concentrations of PRC1 establish a compact higher-order chromatin structure that inhibits ATP-dependent chromatin remodeling and transcription of PcG target genes (3). PRC2 is phylogenetically the most ancient of the PRCs (2) and forms the functional core of the PcG repression machinery. It consists of the four subunits Enhancer of Zeste (E(z)), Suppressor of Zeste 12 (Su(z)12), Extra sex combs (Esc) and Nurf55 (4-7). Histone methyl-transferase activity is associated with the SET domain of E(z), which specifically methylates lysine 27 in histone H3 (H3K27). The third Polycomb Repressive Complex, PhoRC, contains the sequence-specific DNA binding protein Pleiohomeotic (Pho) homologous to the mammalian transcription factor YY1, and the MBT domain protein dSfmbt, which binds mono- and dimethylated lysines of H3 and H4 tails (8).

Nurf55 is a non-catalytic subunit of PRC2 that in vitro is necessary to ensure high-affinity binding of the complex to nucleosomes (7,9). Nurf55 is also found in several other chromatin-modifying complexes including histone acetyltransferase (HAT1) and histone deacetylase (HDAC) complexes, chromatin assembly factor-1 (CAF-1) and the ATP-dependent nucleosome remodeling complexes NURF and NuRD (reviewed in (10)). In addition, Nurf55 forms complexes with histones H3 and H4 and with the essential centromeric variant CenH3 (11). Nurf55 was proposed to act as a recruiting factor that tethers different complexes to their substrate nucleosomes, as well as a histone chaperone that deposits H3-H4 dimers during replication-dependent and independent nucleosome assembly (12). The X-ray structure of Drosophila Nurf55 has recently been determined, alone and in complex with a fragment of histone H4 at 2.9 Å and 3.2 Å, respectively (13). In addition, the structure of the human ortholog RbAp46 bound to a histone H4 peptide has been reported at 2.4 Å resolution (14). Nurf55 forms a 7-bladed β-propeller characteristic for the WD40 family of proteins,
known to be involved in protein-protein interactions (15). The β-propeller is preceded by a long N-terminal helix.

Based on our high resolution (1.75 Å) structure of Nurf55 bound to the first helix of histone H4, we analyze the interactions of Nurf55 with histone H3, histone H4 and the PRC2 component Su(z)12. We identify a cluster of highly conserved negatively charged residues at the top surface of the Nurf55 β-propeller which mediates the binding of the N-terminal tail of histone H3. We also show that the binding pocket at the edge of the Nurf55 β-propeller that binds the first helix of histone H4 is also involved in interaction with Su(z)12.

Experimental Procedures

Protein expression and purification – *Drosophila* Nurf55 (p55) wildtype and mutant proteins were expressed in Sf21 cells as N-terminal, TEV cleavable, His-tagged fusion proteins and purified using standard procedures as detailed in the supplemental data. CD spectra of Nurf55 wildtype and mutant proteins confirmed that the overall structure is preserved in all mutants (see supplemental Fig. S1). FLAG-tagged Su(z)12 and E(z) proteins were expressed separately or co-expressed with wildtype and mutant Nurf55 proteins in Sf21 insect cells and purified using anti-FLAG affinity resin as described (9). Su(z)12 fragments were expressed as TEV-cleavable, GST-fusion proteins in E. coli and purified using standard procedures (see supplemental data). For the binding experiments, histone H3-H4 heterodimers, H426-45 peptide and lysozyme (Sigma) were crosslinked to Dynabeads (Dynal Biotech) according to the manufacturer’s instructions.

Crystalllographic structure determination – For crystallization purified Nurf55 was mixed with the H426-45 peptide at a ratio of 1:3. Best crystals were obtained mixing 1 µl of complex and 1 µl of precipitant solution (35% (v/v) PEG 400, 100 mM MES, pH 6) using the hanging-drop vapour diffusion method. Diffraction data were collected at beamline ID23-2 at the European Synchrotron Radiation Facility (ESRF), Grenoble and were processed and scaled using programs XDS and XSCALE (16). The crystals diffracted to 1.7 Å and belonged to space group P2_1 with one molecule per asymmetric unit. The structure of Nurf55 bound to the H4 peptide was solved by molecular replacement using program Phaser v1.3 (17) with the structure of Nurf55 alone (PDB 3C99 chain A, (13)) as search model. The model was re-built with program Coot v0.4 (18) and refined using program Phenix v1.3 (19) to an R-factor of 17.3% and a free R-factor of 20.5% (supplemental Table 1).

ITC measurements – ITC measurements were performed at 25 °C using a VP-ITC Microcal calorimeter (Microcal, Northampton, USA). All proteins were dialysed overnight against ITC buffer (10 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1 mM β-mercaptoethanol). Lyophilised peptides were resuspended in ITC buffer. Each titration experiment consisted of injecting 10 µl of a 200 µM peptide solution into 2 ml of a 10 µM protein solution at time intervals of around 5 min. Experiments in which the K_D was higher than 10 µM were repeated with 2.5 times higher concentrations of protein and peptide. For competition ITC experiments we used 8-12 µM Nurf55 pre-incubated in the cell with 30-100 µM of competitor peptide (Table 2). To fit an apparent association constant K_D, app we used standard procedures (20) implemented in the MicroCal Origin 7.0 software package.

RESULTS AND DISCUSSION

Binding of Nurf55 to histone H4 - Nurf55 from *Drosophila melanogaster* and its two human homologs RbAp46 and RbAp48 have been reported to bind the first helix of histone H4 (21,22). Using isothermal titration calorimetry (ITC) we found that full-length, recombinant Nurf55 specifically binds to a 20-residue peptide corresponding to the first helix of H4 (hereafter referred to as H4 peptide) with high affinity (K_D = 35 nM, Table 1). In contrast, histone tail peptide H41-15 and a scrambled H426-45 peptide with the same amino acid composition but different primary sequence did not bind to Nurf55. Nurf55 was co-crystallized with this peptide and the structure was solved by molecular replacement at 1.75 Å resolution using the structure of *Drosophila* Nurf55 without peptide as search model (13).

The high resolution of the Nurf55-H4 peptide complex structure (Figs. 1 and 2) allows for a more detailed analysis of the interactions between Nurf55 and H4 peptide than previously possible. The Nurf55 binding surface consists of a hydrophobic moiety formed by the N-terminal helix and residues from blade 6, and a negatively charged moiety mainly formed by residues
protruding from the short helix in the loop connecting two strands in blade 6 (Figs. 1A & 2). Most importantly, Arg39 of the H4 peptide (H4R39, Fig. 2A) forms a network of hydrogen bonds involving two main chain carbonyl groups of Nurf55 (Asp362 and Gly366), the side chain of Gln358 and a water mediated hydrogen bond with Asp365 (Fig. 2). Mutating H4R39 to alanine causes a 3000-fold decrease in the binding affinity to Nurf55 (K_D = 115 μM). Similarly, mutating Gln358 of Nurf55 to alanine resulted in over 500-fold decrease in the affinity to H4 (K_D = 26 μM). Two other arginine residues in the H4 peptide, H4R36 and H4R40, also play important roles in Nurf55 binding. H4R36 forms two hydrogen bonds with Asp362 of Nurf55, and H4R40 forms one direct and one water-mediated hydrogen bond with Asp365. Mutating these two arginines to alanine lowered the affinity to Nurf55 about 20-fold (K_D = 0.87 μM). In contrast, mutating both arginines to lysine only led to about 3-fold weaker binding to Nurf55 (K_D = 0.11 μM) indicating that the positively charged surface of the H4 helix rather than the two arginines is important for binding to Nurf55 (Table 1).

Two aspartate residues of Nurf55, Asp362 and Asp365, that form hydrogen bonds with H4 residues H4R36, H4R39 and H4R40 (see above) contribute to the negative surface charge on one side of the Nurf55 binding pocket (Figs. 1B, 2 & 3A). Mutating both residues to alanine strongly lowered the binding affinity towards the H4 peptide (K_D = 9.6 μM), while mutating them to glutamate, preserving the negative surface charge of the binding pocket, had a much weaker effect (K_D = 0.44 μM). In order to test the contribution of hydrophobic residues to the Nurf55-H4 interaction, residues Leu35, Phe372 and Ile373 of Nurf55 were mutated to serine (Fig. 3A). The triple mutant Nurf55L35S/F372S/I373S binds the H4 peptide with significantly lower affinity (K_D = 15.4 μM).

Interaction of Nurf55 with the N-terminal tail of histone H3 - Binding of Nurf55 to histone H3, histone H4 and to an in vitro reconstituted complex of H3-H4 dimers and tetramers in dynamic equilibrium have been reported (13). Many of the known interactions between histones and other proteins are mediated by N-terminal histone tails, which are poorly structured and accessible for binding in the context of the H3-H4 dimer and the nucleosome. We therefore tested binding of Nurf55 to a 28-residue H3 tail peptide (H31-28) using ITC (Fig. 3A) and found that Nurf55 binds this peptide with low but significant affinity (K_D = 2 μM, Table 1). The shorter peptide H31-15 was bound with the same affinity as the longer peptide H31-28. In contrast, peptide H313-28 was bound with 9-fold reduced affinity, while peptide H36-20 did not bind. Scrambled H31-15 and H1-28 peptides also did not bind Nurf55 further confirming the specificity of the observed interaction (Table 1). Competition experiments show that peptide H31-15 can still bind Nurf55 even when peptide H313-28 is present in excess, while in the reverse experiment the second peptide does not bind Nurf55 (Table 2). We therefore conclude that the H31-15 peptide is the stronger interactor and preferentially occupies the H3 binding site in Nurf55, although peptide H313-28 maintains some binding capacity.

Trimethylation of lysine 4 (K4me3) in the H31-15 peptide strongly reduced binding to Nurf55 (K_D > 500 μM), while the effect is smaller for K9me3 (K_D = 22 μM). In the context of the longer H31-28 peptide the binding affinity for the K4me3 containing peptide is reduced by a factor of three, while longer K9me3 or K27me3 containing H3 peptides bind with similar affinities as the unmodified peptide (Table 1). At present it is unclear why the K9me3 or K27me3 containing H31-28 peptides bind with similar affinities as the unmodified H3 peptides and better than the shorter K9me3 containing H31-15 peptide (Table 1). We speculate that trimethylation of K9 and K27 favors a slightly different conformation of the H31-28 peptide that allows the additional C-terminal residues to contribute to the binding (Table 1). Trimethylation of lysine 4 in histone H3 is generally associated with active chromatin, trimethylation of K9 and K27 with repressive chromatin. The capacity of Nurf55 to bind unmethylated and “repressive” K9me3 and K27me3 containing histone H3 peptides equally well, but also “active” K4me3 containing peptides (although with reduced affinity), might reflect its versatile roles in different chromatin-modifying complexes.

Sequence conservation mapped on the surface of Nurf55 revealed a patch of highly conserved residues, many of them negatively charged, on the top of the β-propeller surrounding the central channel (Figs. 1B & C). This site is commonly used for peptide binding in WD40 proteins (15) and could conceivably serve as the interaction surface for the positively charged H3 tail. To test this hypothesis double,
triple and five-fold mutations were introduced in Nurf55 changing conserved acidic residues in this region into uncharged or basic residues (Fig. 3C). CD spectra confirmed that the overall structure of the mutants was preserved (see supplemental Fig. S1). The mutant proteins were tested for histone H3 binding using ITC. While one mutant (NurfD322N/E323K) bound the peptide H31-28 with 11-fold reduced affinity, the two other mutants (NurfE235Q/D252K/E279Q and NurfE235Q/D252K/E279Q/D322N/E323K) no longer showed binding to the H3 peptide confirming that we correctly identified the interaction site (Table 1). Mutations in the H4 binding pocket (Nurf55D362A/D365A and Nurf55L35S/F372S/I373S) that interfere with H4 binding did not change the affinity for the H3 tail. Similarly, mutations of the H3 binding pocket (D322N/E323K and E235Q/D252K/E279Q) did not affect the affinity for the H4 peptide (Table 1). Competition experiments also show that the H3 peptide binds to Nurf55 with similar affinity in the presence of an excess of the H4 peptide and similarly H4 binding is not affected by an excess of the H3 peptide (Table 2). Nurf55 therefore uses two independent binding sites for interactions with the H3 tail and H4 helix 1.

Binding of the H3-H4 heterodimer to Nurf55 - The mechanism by which Nurf55 binds histones H3 and H4 is poorly understood. H4 helix 1 is not accessible for binding Nurf55 in the conformation that it adopts in nucleosomes (23), in H3-H4 dimers or tetramers or in H3-H4 dimers bound to the histone chaperone Asf1 (24,25). Therefore, the H3-H4 dimer must undergo a structural rearrangement upon binding to Nurf55 making helix 1 of histone H4 accessible for interaction (Song et al, 2008). How Nurf55 initially recruits the H3-H4 dimer is also unclear.

Pull-down experiments using site-directed Nurf55 mutants of the H3 (E235Q/D252K/E279Q) or the H4 binding site (D362A/D365A) with significantly lower affinities for the respective peptides (Table 1) showed reduced but not completely abolished binding to H3-H4 dimers (Fig. 3D) in agreement with the existence of two independent binding sites. The H4 binding site mutant bound weaker to the H3-H4 dimer compared to the H3 binding site mutant (Fig. 3D) consistent with the higher affinity of the H4 peptide compared to the H3 peptide to Nurf55 (Table 1).

The presence of two independent Nurf55 binding sites for the H3 N-terminal tail and for H4 helix 1 argues against a sequential binding mechanism. Nevertheless, H3 tail binding presumably is the more rapid initial targeting step, bringing Nurf55 in proximity to the H3-H4 dimers or tetramers, while H4 peptide binding is probably slower as it requires a conformational change that makes helix 1 of histone H4 accessible for binding into the groove at the side of the β-propeller (Fig. 3B). Helix 1 of histone H4 is located opposite to the interface of the H3-H4 dimer with the second dimer in the H3-H4 tetramer. Binding of Nurf55 to this helix would therefore not interfere with the formation of H3-H4 tetramers. In vitro reconstituted Nurf55/H3-H4 complex predominantly migrates like a complex of ~70 kDa in size exclusion chromatography that would correspond to one molecule of Nurf55 bound to a H3-H4 dimer (75.3 kDa) (supplemental Fig. S2A). Analytical ultracentrifugation also shows the presence of a 75 kDa complex that could correspond to Nurf55 bound to H3-H4 dimers, but also complexes that could correspond to H3-H4 tetramers bound to one Nurf55 (102 kDa) or two Nurf55 molecules (150 kDa) and even higher molecular species (supplemental Fig. S2B). In addition, Nurf55 could also bind H3-H4 dimers already bound by other histone chaperones as it has been suggested for Asf1/CIA that binds histone H3-H4 dimers opposite of the Nurf55 binding site (14).

Interaction of Nurf55 with PRC2 component Su(z)12 - In the context of PRC2, Nurf55 interacts with Su(z)12 (9,26). To explore whether this interaction involves the same binding pocket which interacts with the H4 peptide, we co-expressed FLAG-tagged Su(z)12 with wildtype Nurf55 and two Nurf55 mutants (Nurf55D362A/D365A, Nurf55L35S/F372S/I373S) that change the H4 peptide binding pocket. Wildtype Nurf55 was co-purified with FLAG-Su(z)12 in a 1:1 stoichiometry (Fig. 4). In contrast, binding of both Nurf55 mutants to Su(z)12 was dramatically reduced (Fig. 4), indicating that residues involved in H4 peptide binding are also important for binding the PRC2 component Su(z)12. In accordance with the results of Ketel et al. (2005) we did not observe a direct interaction between Nurf55 and the PRC2 component E(z) (data not shown).

Full-length protein Su(z)12 can be expressed in insect cells, but tends to form soluble aggregates when expressed and purified individually. To better characterize the Nurf55 interacting region of Su(z)12, we expressed nine ~100-residue constructs spanning the entire
Su(z)12 protein (Fig. 5A). The constructs were chosen to preserve predicted domains and secondary structure elements of Su(z)12 and were expressed in E. coli as N-terminal GST-fusion proteins (Fig. 5B). All constructs were tested for their ability to bind Nurf55 in GST pull-down assays. The N-terminal construct corresponding to the first 100 residues (referred to as Su(z)12-1) showed binding to Nurf55 (Fig. 5B, lower panel). Using ITC the Su(z)12-1 construct was found to reproducibly bind Nurf55 although with low affinity (KD = 24 µM, Table 1). In contrast, no interaction was observed between Su(z)12-1 and two Nurf55 mutants of the H4 binding pocket (NurfD362A/D365A and NurfL35S/F372S/I373S) using ITC and GST pull-down assays (supplemental Fig. S3A), consistent with the observation that these mutants do not bind full-length Su(z)12 protein (Fig. 4A).

Competition experiments provided additional support for overlapping Nurf55 binding sites for Su(z)12 and histone H4, but not histone H3. In the presence of an excess of the H4 peptide (Table 2), the binding affinity of Nurf55 for Su(z)12-1 was reduced. Consistent with these results, we found that increasing concentrations of Su(z)12-1 abrogate binding of Nurf55 to the H426-45 peptide cross-linked to beads in pull-down experiments (Fig 5C). At a ratio Su(z)12-1:Nurf55  50:1 (mol/mol), Nurf55 is no longer bound to the H4 peptide. In contrast, an excess of the H3 peptide did not significantly change the binding affinity for Su(z)12-1. In the reverse experiments, an excess of Su(z)12-1 did not change the affinity for the H3 peptide (Table 2).

While the N-terminal moiety of Drosophila Su(z)12 is poorly conserved subsequent residues 50-100 of Su(z)12 are predicted to form three α-helices with the second helix (residues 66-80) containing a cluster of arginines (R70, R73 and R75) separated by hydrophobic residues (Fig. 5D). This region remarkably resembles H4 helix 1, where three arginines (R36, R39 and R40) also separated by hydrophobic residues play a crucial role in binding to Nurf55. To test whether these arginines of Su(z)12 are involved in binding to Nurf55, they were mutated to alanine and the mutant protein Su(z)12-1R70A/R72A/R75A was tested for binding to Nurf55 in a pull-down assay. No binding to Nurf55 was observed (supplemental Fig. S3B), which suggests that at least one of the three arginine residues is required for binding. However, a short peptide spanning only residues corresponding to the second predicted helix of Su(z)12 (Su(z)12-163-81, Fig. 5D) did not bind to Nurf55 in ITC experiment. Extending this helix towards the N- and C-terminal ends (peptides Su(z)12-145-80, Su(z)12-163-96) did not increase the binding affinity which suggests that an even more extended region of Su(z)12 might be required for Nurf55 binding.

Concluding remarks - Nurf55 uses a newly identified binding site at the top of the β-propeller for interacting with histone H3 and overlapping binding sites at the propeller edge for binding H4 helix 1 and Su(z)12. In PRC2, Nurf55 and Su(z)12 together provide interaction surfaces to tether the complex to nucleosomes, while Nurf55 or Su(z)12 alone are not sufficient for binding (9). Nucleosome binding by PRC2 presumably involves the histone H3 binding site of Nurf55, while in the PRC2 complex histone H4 and Su(z)12 compete for overlapping binding sites. Whether in the PRC2 complex Nurf55 binds only Su(z)12 or both, Su(z)12 and histone H4, at different stages of the catalytic process will require further investigation.

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**FIGURE LEGENDS**

**Fig. 1.** Overview of the Nurf55-H4-peptide structure. A. Front and top view (turned by 90° around a horizontal axis) of the Nurf55-H4-peptide structure. Nurf55 is depicted in marine and the H4 peptide in yellow. Numbers in the lower left panel label the blades of the \( \beta \)-propeller. B. Electrostatic potential mapped on the surface of Nurf55. Red depicts acidic patches, white neutral patches and blue basic patches (overall range from -30 kT to +30 kT). C. Sequence conservation mapped on the surface of Nurf55. Completely conserved residues are indicated in violet, residues >80% conserved are indicated in purple and >60% are indicated in light blue.

**Fig. 2.** Overview of the Nurf55-peptide interaction. A. Stereo view of polar interactions between Nurf55 and the H4 peptide. Nurf55 and the peptide are depicted as blue and yellow ribbons, respectively. Interacting residues enclosed by a 2Fo-Fc electron density map at 1.0 \( \sigma \) are depicted in stick representation. B. Schematic representation of the Nurf55-peptide interactions. The three helices and blade 6 forming the binding pocket, as well as blade 7 are depicted in blue, the connecting residues between blade 7 and blade 6 are depicted as blue dashed line and the H4-peptide in orange. Hydrophobic interactions are drawn in black, hydrogen bonds in red and water-mediated hydrogen bonds in red dashed lines. The hydrogen bond network shown in (C) is indicated with green lines. C. Close-up view of the hydrogen bond network between the NH2 group of R39, the carboxamide of Q358, and the main chain carbonyls of D362 and G366. The network is indicated with green dashed lines.

**Fig. 3.** Interaction of Nurf55 with histones H3 and H4. A. Secondary structure elements of histones H3 and H4 from *Drosophila melanogaster*. Peptides H3_{1-28}, H3_{1-15}, H3_{6-20}, H3_{13-28} and H4_{26-45} used for ITC measurements and pull-down assays are indicated. Peptides containing trimethylated K4, K9 and K27 residues as indicated by asterisks were tested for Nurf55 binding. The sequences of both peptides are given. B. Side view of the Nurf55 \( \beta \)-propeller. Residues mutated in the H4 binding groove are depicted. C. Top view of Nurf55 with residues where residues mutated in the H3 tail binding site are indicated. D. Site-directed Nurf55 mutants of the H3 binding site (E235Q/D252K/E279Q) and the H4 binding site (D362A/D365A) are named NmH3 and NmH4, respectively. Wild-type Nurf55 or Nurf55 mutants NmH3 and NmH4 were pulled down by histones H3-H4 dimers cross linked to Dynabeads. Lysozyme was also cross linked to beads and was used as a negative control. In each experiment input lane (i) contains 1/10 of the protein used in each of the pull-down assays, lane (b) corresponds to the pulled-down proteins.

**Fig. 4.** Interaction of Nurf55 with a PRC2 component Su(z)12. A. Pull-down experiments of wildtype and mutant Nurf55 proteins (Nurf55_{D362A, D365A} and Nurf55_{L355S,F372S,F373S}) with FLAG-tagged, full-length Su(z)12. B. Western blot of cell lysates with anti-Nurf55 antibodies demonstrates that wildtype and mutant Nurf55 proteins were expressed at comparable levels.

**Fig. 5.** Interaction of Nurf55 with the N-terminus of Su(z)12. A. Full-length Su(z)12 and fragments Su(z)12-1 to 9 were expressed in *E. coli* as N-terminal GST-fusion proteins. Constructs were designed to preserve the predicted zinc finger (residues 411-434), predicted secondary structure elements and the VEF homology box (residues 527-603, (27)). The results shown in Fig. 5B are summarized in the lane “Interaction with Nurf55”. B. Purification of Su(z)12 fragments using glutathione-base affinity purification (upper panel) and proteins cleaved from the glutathione beads and analyzed by Western blot with anti-Nurf55 antibodies (lower panel). Only full-length Su(z)12 and Su(z)12-1 fragment (residues 1-100) bind Nurf55. The low signal of full-length Su(z)12 compared to bacterially expressed Su(z)12-1 presumably results from partial aggregation of this protein. C. Fixed amounts of Nurf55 in the absence or in the presence of increasing concentrations of Su(z)12-1 (0.5x, 1x, 10x, and 50x excess compared to Nurf55 (mol/mol)) were pulled down by H4_{26-45} peptide fixed to Dynabeads. In each experiment, the input lane (i) contains 1/10 of the protein used in each of the pull-down assays and...
lane (b) corresponds to the bound material. D. Alignment of residues 51 to 100 of Drosophila Su(z)12 with the corresponding regions in Xenopus laevis and human orthologues. Predicted secondary structure elements in Su(z)12 are depicted. Helix 2 of Su(z)12 contains a similar pattern of conserved arginine residues (blue) and hydrophobic residues (red) as H4 helix 1.
Table 1. Binding affinities of wildtype and mutant Nurf55 to H4 and H3 peptides and Su(z)12-1

| Protein | Peptide<sup>a</sup> | KD (µM)<sup>b</sup> | N<sup>c</sup> | ΔG<sup>°</sup> (kcal/mol) | ΔH<sup>°</sup> (kcal/mol) | ΔS<sup>°</sup> (kcal/mol) |
|---------|--------------------|-------------------|--------|-----------------|-----------------|-----------------|
| Wt Nurf55 | H4<sub>26-45</sub> | 0.035±0.001 | 1.01 | -10.3 | -18.9 | -8.6 |
|         | H4-R39A | 115±8 | 1.00 | -5.4 | -18.5 | -13.1 |
|         | H4-R36A,R40A | 0.87±0.04 | 0.97 | -8.3 | -14.8 | -6.5 |
|         | H4-R36K,R40K | 0.11±0.01 | 0.97 | -9.5 | -15.5 | -6.0 |
| Mutant - H4/Su(z)12 site | H4<sub>26-45</sub> (scrambled)<sup>c</sup> | > 500 | - | - | - | - |
|         | H4<sub>1-15</sub> | > 500 | - | - | - | - |
|         | H4<sub>1-15</sub> (scrambled)<sup>c</sup> | > 500 | - | - | - | - |
| Mutant - H3 site | D362A/D365A | 9.6±0.8 | 0.96 | -6.9 | -19.0 | -12.2 |
|         | D362E/D365E | 0.44±0.03 | 1.06 | -8.7 | -9.5 | -0.9 |
|         | Q358A | 26±2 | 1.00 | -6.3 | -17.3 | -11.0 |
|         | L35S/F372S/1373S | 15.4±1.4 | 1.00 | -6.6 | -15.0 | -8.4 |
| H3<sub>1-28</sub>: ARTK<sub>q</sub>QTARK<sub>q</sub>STGGKAPRKQLATKAARK<sub>27</sub>S | Wt Nurf55 | H3<sub>1-28</sub> | 1.6±0.1 | 1.11 | -7.9 | -14.5 | -6.6 |
|         | H3<sub>1-28</sub>K4me3 | 6.4±0.6 | 0.83 | -7.1 | -18.0 | -10.9 |
|         | H3<sub>1-28</sub>K9me3 | 1.22±0.06 | 1.19 | -8.1 | -15.8 | -7.7 |
|         | H3<sub>1-28</sub>K27me3 | 0.69±0.05 | 1.11 | -8.4 | -15.0 | -6.5 |
| Mutant - H3 site | H3<sub>1-15</sub> | 1.4±0.1 | 1.13 | -8.0 | -14.5 | -7.2 |
|         | H3<sub>1-15</sub>K4me3 | 6.4±0.6 | 0.83 | -7.1 | -18.0 | -10.9 |
|         | H3<sub>1-15</sub>K9me3 | 1.22±0.06 | 1.19 | -8.1 | -15.8 | -7.7 |
|         | H3<sub>1-15</sub>K27me3 | 0.69±0.05 | 1.11 | -8.4 | -15.0 | -6.5 |
|         | H3<sub>1-15</sub> (scrambled)<sup>c</sup> | > 500 | - | - | - | - |
|         | H3<sub>1-15</sub> (scrambled)<sup>c</sup> | > 500 | - | - | - | - |
| Su(z)12-1 construct | Wt Nurf55 | Su(z)<sub>12-1</sub><sup>a</sup> | 24±1 | 1.00 | -6.3 | -21.9 | -15.6 |
| Mutant - H4/Su(z)12 site | D362A/D365A | > 500 | - | - | - | - |
|         | L35S/F372S/1373S | > 500 | - | - | - | - |

a) H3 and H4 peptides were chemically synthesized. Construct Su(z)12-1 comprises Drosophila Su(z)12 residues 1 to 100 as shown in Figure 5A and was expressed and purified as described in the supplemental information.
b) Errors of the KD are fitting errors using the data evaluation program.
c) The following peptides were used as internal control: H4<sub>26-45</sub>(scrambled): PKGIRTAARGQVRIIRLK, H4<sub>1-15</sub>: TGRGGKGGKGLGKGGA, H4<sub>1-15</sub>(scrambled): KGKGGAKRGGLGGT, H3<sub>1-28</sub>(scrambled): QTALTSTGRTKQSAAKPKGKAAR, H3<sub>1-15</sub> (scrambled): TRSTAAQTKGGRKAK.
Table 2. Competitive binding of the H3, H4 peptides and the Su(z)12-1 construct

| Competition experiment | Nurf55 (μM) | Peptide with fixed conc. | Concentration (μM) | Peptide with variable conc. | $K_D,\text{ app}^a$ (μM) |
|------------------------|-------------|--------------------------|--------------------|-----------------------------|--------------------------|
| $H_3^{13-28} + H_3^{1-15}$ | 8.8 | $H_3^{13-28}$ | 33 | $H_3^{1-15}$ | 4.5 ± 0.3 |
| $H_3^{1-15} + H_3^{13-28}$ | 8.8 | $H_3^{1-15}$ | 33 | $H_3^{13-28}$ | > 250 |
| $H_4^{26-45} + H_3^{1-28}$ | 8.3 | $H_4^{26-45}$ | 68 | $H_3^{1-28}$ | 0.98 ± 0.04 |
| $H_3^{1-28} + H_4^{26-45}$ | 8.3 | $H_3^{1-28}$ | 66 | $H_4^{26-45}$ | 0.09 ± 0.01 |
| $H_3^{1-28} + Su(z)12-1$ | 7.8 | $H_3^{1-28}$ | 95 | $Su(z)12-1$ | 33 ± 4 |
| $Su(z)12-1 + H_3^{1-28}$ | 11.6 | $Su(z)12-1$ | 100 | $H_3^{1-28}$ | 2.21 ± 0.25 |
| $H_4^{26-45} + Su(z)12-1$ | 7.9 | $H_4^{26-45}$ | 95 | $Su(z)12-1$ | 110 ± 3 |
| $Su(z)12-1 + H_4^{26-45}$ b) | 11.6 | $Su(z)12-1$ | 100 | $H_4^{26-45}$ | n.d. |

a) Errors of the $K_D,\text{ app}$ are fitting errors using the data evaluation program.
b) No $K_D$ could be determined presumably because of precipitation of Nurf55.
Figure 3

A

Histone H3

H3 peptide

ARTKOTARKOTGKKAPRKQLATKAARKS

Histone H4

H4 peptide

IQGITKPAIRRLARRGGV

B

C

D

Lysozyme

H3/H4

|    | Nur55 | NmH3 | NmH4 | Nur55 | NmH3 | NmH4 |
|----|-------|------|------|-------|------|------|
| i  | b     | i    | b    | i     | b    | i    |

Nur55

H3

H4
Figure 4

A

B
Figure 5

A

| Su(z)12:  | 1 | 411 | 34 | 527 | 603 | 900 | + |
| Su(z)12-1: | 1 | 100 |   |     |    |     | + |
| Su(z)12-2: | 99 | 200 |   |     |    |     | - |
| Su(z)12-3: | 201| 300 |   |     |    |     | - |
| Su(z)12-4: | 301| 400 |   |     |    |     | - |
| Su(z)12-5: | 401| 500 |   |     |    |     | - |
| Su(z)12-6: | 497| 607 |   |     |    |     | - |
| Su(z)12-7: | 591| 700 |   |     |    |     | - |
| Su(z)12-8: | 701| 800 |   |     |    |     | - |
| Su(z)12-9: | 801| 900 |   |     |    |     | - |

B

Coomassie Blue-Stained Gel

IB : anti-Nurf55

C

Su(z)12-1

| Su(z)12-1 | 0 | 0.5 | 1 | 10 | 50 |
| i | b | i | b | i | b | i | b | i | b |

D

D. melanogaster

HQQEQLFLQAFEKPTQIYRYLNRHETNPILNRTLTSMKERMSPNNKK

X. laevis

IQADHELFLQAFEKPTQIYFLRTNLIAPIFLHRTLYMSHRNSRSNAR

H. sapiens

VQADHELFLQAFEKPTQIYFLRTNLIAPIFLHRTLYMSHRNSRTNIK

Su(z)12 helix 2: TQIYRYLRNPRHE

Histone H4 helix 1: PAIRLARRGGV
Chromatin-modifying complex component Nurf55/p55 associates with histones H3, H4 and Polycomb Repressive Complex 2 subunit Su(z)12 through partially overlapping binding sites

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