Identification of Functionally Conserved Regions in the Structure of the Chaperone/CenH3/H4 Complex

Jingjun Hong¹, Hanqiao Feng¹, Zheng Zhou², Rodolfo Ghirlando³ and Yawen Bai¹

¹ - Laboratory of Biochemistry and Molecular Biology, National Cancer Institute, NIH, Bethesda, MD 20892, USA
² - National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
³ - Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA

Correspondence to Yawen Bai: yawen@helix.nih.gov
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Abstract

In eukaryotes, a variant of conventional histone H3 termed CenH3 epigenetically marks the centromere. The conserved CenH3 chaperone specifically recognizes CenH3 and is required for CenH3 deposition at the centromere. Recently, the structures of the chaperone/CenH3/H4 complexes have been determined for Homo sapiens (Hs) and the budding yeasts Saccharomyces cerevisiae (Sc) and Kluyveromyces lactis (Kl). Surprisingly, the three structures are very different, leading to different proposed structural bases for chaperone function. The question of which structural region of CenH3 provides the specificity determinant for the chaperone recognition is not fully answered. Here, we investigated these issues using solution NMR and site-directed mutagenesis. We discovered that, in contrast to previous findings, the structures of the Kl and Sc chaperone/CenH3/H4 complexes are actually very similar. This new finding reveals that both budding yeast and human chaperones use a similar structural region to block DNA from binding to the histones. Our mutational analyses further indicate that the N-terminal region of the CenH3 α2 helix is sufficient for specific recognition by the chaperone for both budding yeast and human. Thus, our studies have identified conserved structural bases of how the chaperones recognize CenH3 and perform the chaperone function.

During mitosis, paired sister chromatids are segregated equally into daughter cells by microtubules that are attached to the mitotic spindle at the centromere through a multi-protein complex called the kinetochores.

A conserved conventional H3 variant termed CenH3 (Cse4 in budding yeast and CENP-A in human) epigenetically marks the centromere and supports the formation of the kinetochore complex.

The structures of three of the chaperones in complex with the corresponding CenH3/H4 histones of Saccharomyces cerevisiae (Sc), Kluyveromyces lactis (Kl), and Homo sapiens (Hs) have been determined at atomic resolution (Fig. 1a–c). The structures of three of the chaperones in complex with the corresponding CenH3/H4 histones of Saccharomyces cerevisiae (Sc), Kluyveromyces lactis (Kl), and Homo sapiens (Hs) have been determined at atomic resolution (Fig. 1a–c). In each of the structures, the chaperone forms a heterotrimer with CenH3/H4 and binds to the histone sites that interact with DNA in the canonical nucleosome structure (Fig. 1a–c), providing a structural mechanism for its chaperone function, whereby binding between the chaperone and histones prevents the direct interaction between histones and DNA.

Despite the common structural features of the chaperone–histone heterotrimers, surprisingly large differences are apparent in the three structures (Fig. 1a–c). For example, a large portion of the Cse4/H4 structure in the Sc CenH3/Cse4/H4 complex is unstructured, whereas the CenH3/H4 histones in the Kl and Hs complexes have a completely ordered histone fold. Also, the N-terminal region of the Kl Scm3 forms a β-hairpin and occupies the DNA-binding sites of the nucleosome histones, while in contrast, the corresponding regions in the Sc Scm3 and Hs HJURP are unfolded. The structural differences between the Sc and Kl Scm3/Cse4/H4

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complexes are particularly striking, especially because the corresponding histones and chaperones have a very high sequence homology (Fig. 1d and Ref. 12). These differences lead to different conclusions regarding the structural basis of the chaperone functions, namely, that the Sc Scm3 uses the C-terminal helix and the loop region that follows the N-terminal helix to prevent binding of DNA to the L1 loop of Cse4 and the L2 loop of H4, while the N-terminal β-hairpin of the Kl Scm3 prevents binding of DNA to the L2 loop of Cse4. There has been much speculation about the possible causes for the structural differences of the Sc and Kl Scm3/Cse4/H4 complexes. Cho and Harrison suggested that the deletion of the α1 helix of H4 and the use of a single-chain model may have led to an incorrect NMR structure of the Sc Scm3/Cse4/H4 complex.12 On the other hand, Zhou et al. pointed out that the shorter Scm3 construct used to determine the structure of the Kl/Scm3/Cse4/H4 complex, together with crystal packing, may have conduced to form the completely folded histone-fold structure.14,15

In addition, it is currently debated which structural region in CenH3 is the determinant for specific recognition by the chaperone. We have previously shown that four Sc Cse4-specific residues in the N-terminal region of the α2 helix are necessary and sufficient for Scm3 recognition.11 In contrast, it was proposed that a single CENP-A-specific residue in the α1 helix, which is located outside of the centromere-targeting domain of CENP-A (CATD, loop 1 and helix 2), is the primary specificity determinant.13,16 More recently, it was suggested that the combination of the two C-terminal residues in the α2 helix of CENP-A with either one of the two residues in the N-terminal portion of the α2 helix or the L1 loop is sufficient for CENP-A recognition by HJURP.17

Here, we have investigated both issues using solution NMR and site-directed mutagenesis methods. We find that the solution structure of the Kl/Scm3/Cse4/H4 complex is similar to that of the Sc complex, and the functionally important structural regions in the chaperone/CenH3/H4 complex are largely conserved.

Fig. 1. Comparison of the three structures of CenH3/H4 histones in complex with the homologous domains of their chaperones.14 (a) Sc Scm3/Cse4/H4. (b) Kl Scm3/Cse4/H4. (c) Hs HJURP/CENP-A/H4. The broken lines indicate unfolded regions. The transparent ovals (magenta) indicate the regions that block DNA binding to the histones. In (a) to (c), the chaperone, CenH3, and H4 are in magenta, cyan, and green, respectively. (d) The amino acid sequences of the CenH3/H4 binding domains of the chaperones. Continuous lines show folded regions and secondary structures in the chaperone/CenH3/H4 complexes, whereas the broken lines represent unstructured regions. Conserved residues between Sc and Kl Scm3 are shown in magenta while non-conserved residues are shown in black. For Hs HJURP, conserved residues between HJURP and Scm3 are shown in magenta while non-conserved residues are shown in black.
Results

Solution conformation of the chaperone/CenH3/H4 complex

To determine whether the shorter Scm3 construct and crystal packing could affect the Kl Scm3/Cse4/H4 structure, we examined this complex at pH 5.6 (physiological condition for budding yeast). We used a longer Kl Scm3 that includes the additional C-terminal region corresponding to the unfolded loop and the αC helix in the NMR structure of the Sc Scm3/Cse4/H4 complex (Fig. 1d). This Kl Scm3/Cse4/H4 complex has a molecular mass larger than 30 kDa and precipitates at temperatures higher than 30 °C, prohibiting a full structural determination by NMR. Therefore, we focused on the identification of the unfolded regions of the complex for which NMR signals are readily observable at room temperature due to fast local dynamic motions. Indeed, we observed a large number of cross peaks in the 1H-15N heteronuclear single quantum correlation (HSQC) spectra (Fig. 2a and b), indicating that many residues have an unfolded conformation. We then used 15N13C-labeled Kl Scm3 chaperone and unlabeled Cse4/H4 histones, and vice versa, to assign the backbone chemical shifts of the observable cross peaks in the 1H-15N HSQC spectra. The difference between the measured values and those of random coils for the Cα chemical shifts are between −1 and +1 for most of the residues (Fig. 2c–e). No consecutive three residues have values larger than +1 or smaller than −1, indicating that the observed residues are indeed unfolded.

The unfolded residues are in the regions of 43–57 and 111–129 of Kl Scm3, 160–183 of Kl Cse4, and 30–46 of Kl H4 (Fig. 2f), which either form folded structures or are absent in the crystal structure of the Kl Scm3/Cse4/H4 complex. For example, Kl Scm3 residues 44–57 form the N-terminal β-hairpin while Scm3 residues 116–129 are absent (Fig. 1b); Cse4 residues 160–183 form the last turn of the α2 helix, the L2 loop, and the α3 helix; H4 residues 31–42 form the a1 helix. In contrast, these regions correspond to those that are unfolded in the Sc Scm3/Cse4/H4 NMR structure. Kl Scm3 regions 59–103 and 131–139, Cse4 regions 110–122 and 132–159, and H4 regions 48–77 and 81–98 are not

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**Fig. 2.** Unstructured regions in the three-chain Kl Scm3/Cse4/H4 complex at pH 5.6. (a) 1H-15N HSQC spectrum of Scm3 in the complex. (b) 1H-15N TROSY spectra of Cse4 and H4 in the complex. Assignments are in cyan for Cse4 and in green for H4. (c) Cα chemical shift deviations from random-coil values for Scm3. (d and e) Cα chemical shift deviations from random-coil values for Cse4 and H4, respectively. The corresponding secondary structural regions in the crystal structure are shown. (f) Mapping of the unfolded regions (red) of the Scm3/Cse4/H4 complex at pH 5.6 onto the crystal structure. (g) Analytical ultracentrifugation analysis of the Scm3/Cse4(L158G/L159G/L161G/H162G)/H4 complex at pH 5.6 and a loading concentration of 35 μM. A single species at 2.61 S is observed, having an estimated molecular mass of 33.0 kDa, consistent with a heterotrimeric complex.
observable by NMR, suggesting that they have folded structures. In the crystal structure of the KlScm3/Cse4/H4 complex, KlScm3 residues 59–91 form the αN helix, while KlScm3 residues 92–103 form an irregular structure and have no interactions with the histone proteins. The KlScm3 residues 131–139 are absent in the crystal structure whereas the corresponding residues in ScScm3 form the αC helix in the NMR structure of the ScScm3/Cse4/H4 complex. The αN helix of the KlScm3 appears to be longer than that of ScScm3 by two helical turns at the N-terminal region. We found at lower temperature (25 °C) that the corresponding region in ScScm3 also folds to an α-helical conformation in the complex (Fig. S1) (the NMR structure of the ScScm3/Cse4/H4 complex was determined at 35 °C), indicating that temperature plays a role in the formation of its structure. In addition, mutation of four residues (L158, L159, L161, and H162) in the C-terminal region of the α2 helix of KlCse4 in the crystal structure to Gly did not prevent the formation of a heterotrimeric complex with the KlScm3 and H4 (Fig. 2f and g), indicating that this region is not essential for recognition by KlScm3. Thus, our results reveal that the KlScm3/Cse4/H4 complex has similar unfolded regions as the solution structure of the ScScm3/Cse4/H4 complex (Fig. 1a).

We also used NMR to examine the human HJURP/CENP-A/H4 complex at both pH 5.6 and pH 7.0. We found that the α1 helix of H4 becomes unstructured at pH 5.6, whereas CENP-A remains fully folded except for the last three C-terminal residues (Fig. S2). However, at pH 7.0, only HJURP residues 2–10 and 76–81 and H4 residues 22–30 are unstructured (Fig. S2), consistent with the crystal structure.13

**Structural determination of the partially unfolded Cse4/H4 dimer**

In our previous structural determination of the ScScm3/Cse4/H4 complex, we showed that the free ScCse4/H4 dimer is partially unfolded.11 The unfolded regions include the residues corresponding to those in the C-terminal region of the α2 helix, the L2 loop, and the α3 helix of Cse4 and the α1 helix of H4 within the histone fold. Here, we also examined the KlCse4/H4 complex and found that it is as partially unfolded as the ScCse4/H4 complex (Fig. S3). In order to determine whether the folded region of the ScCse4/H4 dimer has a structure similar to that in the ScScm3/Cse4/H4 complex, we determined its structure at pH 5.6 and 35 °C using a single-chain model (Fig. 3, Fig. S4, and Table S3). In this model,

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**Fig. 3.** NMR structure of the folded region in the single-chain ScCse4/H4 dimer at pH 5.6. (a and b) Overlay of the folded region of the ScCse4/H4 dimer from 10 calculated low-energy structures. (c and d) Comparison of the folded regions of Cse4/H4 in the dimer and in the ScCse4/H4 trimer complex. (e and f) Comparison of the folded regions of Cse4/H4 in the dimer and those of H3/H4 in the nucleosome structure. The structures are aligned on H4.
the C-terminal region of the α2 helix of Cse4 is linked to the N-terminal region of the α2 helix of H4 through a six-residue linker that includes a thrombin protease-cutting site (Table S2). The folded regions of the histones have RMSDs of 0.2 Å for the backbone and 0.8 Å for full atoms. A comparison of the 1H–15N HSQC spectra of the unlinked Sc Cse4/H4 dimer (produced by thrombin cleavage) and that of the single-chain model shows that the linker has little effect on the chemical shifts of the folded regions (Fig. S4), indicating that the structure of the folded region in the Sc Cse4/H4 dimer is not altered in the single-chain model. This conclusion is further supported by the observation that the distance between the N-terminus of the α2 helix of H4 and the C-terminus of the α2 helix of Cse4 in this dimer is longer than that of the corresponding H4 and H3 in the nucleosome structure. We find that the positions of the α1 and α2 helices of Cse4 in the free Cse4/H4 dimer deviate from those in the ScScm3/Cse4/H4 complex (Fig. 3c and d). In addition, the α2 helix of H4 in the free Cse4/H4 dimer does not align well with the corresponding region of H4 in the canonical nucleosome structure (Fig. 3e and f).

Equilibrium between the Cse4/H4 dimer and the (Cse4/H4)2 tetramer

We next investigated the intrinsic properties of the Cse4/H4 complex in the absence of Scm3 at different salt concentration and pH values. At pH 5.6 [50 mM 4-morpholineethanesulfonic acid (Mes)] without salt, velocity sedimentation experiments show that the Kf and Sc Cse4/H4 dimers are dominantly populated in solution (Fig. 4a and Fig. S5a). Larger molecular weight species became more populated as salt concentration or pH was increased (Fig. 4b–d and Fig. S5b–d). This additional species has an apparent molecular weight larger than that of the Cse4/H4 dimer but smaller than or close to that of the (Cse4/H4)2 tetramer (Fig. 4b and c and Fig. S5b and c), likely representing a (Cse4/H4)2 tetramer that is in fast exchange with the Cse4/H4 dimer. At pH 7.4 and in the presence of 150 mM NaCl, the (Cse4/H4)2 tetramer seems to be in equilibrium with a species that has a larger molecular weight as indicated by a broader peak and larger S20,w. In contrast, human CENP-A and H4 predominantly form a CENP-A/H4 dimer at pH 4.5 (50 mM NaAc) and a (CENP-A/H4)2 tetramer at pH 5.6 in the absence of salt (Fig. S5e and f). Thus, around neutral pH, the (CenH3/H4)2 tetramer is the predominant form when chaperone amount is limiting and could be incorporated into ectopic sites in the chromosome.21,22 In budding yeast, an E3 ubiquitin ligase (Psh1) targets the free Cse4 for proteolysis by binding to the CATD region of Cse4,21,22 suggesting that Psh1, like Scm3, may also dissociate the (Cse4/H4)2 tetramer.

Equilibrium between the two-chain Cse4/H4 dimer and the (Cse4/H4)2 tetramer. Velocity sedimentation analyses of the Kf/Cse4/H4 complexes at different pH and salt concentrations. (a) pH 5.6 and 0 mM NaCl. (b) pH 5.6 and 150 mM NaCl. (c) pH 6.5 and 150 mM NaCl. (d) pH 7.4 and 150 mM NaCl. Broadening of the peak indicates the exchange of different oligomers within the time scale of the sedimentation process. In all cases, the species at ~2.0 S has a best-fit molar mass consistent with a Cse4/H4 dimer. The faster sedimenting species represents the reaction boundary describing the fast exchange of the tetramer and the dimer, except in the case of (d) where the broader and faster sedimenting peak is resolved into tetramers and possibly hexamers.
Mutational analysis of CenH3 recognition by the chaperones

We have previously shown that four Sc Cse4-specific residues (M181, M184, A189, and S190) in the α2 helix N-terminal region are necessary and sufficient for Scm3 recognition (Fig. 5a). These four residues are located within the CATD required for CenH3 deposition in the centromere. As these four residues are fully conserved in Kl Cse4, we used them to replace the corresponding residues in the Kl H3 and found that His-tagged Kl Scm3 can also recognize the Kl H3(S88M/G91M/S96A/V97S)/H4 dimer (Fig. 5b).

In contrast, only one residue (Sc Cse4 A189 or Kl Cse4 A144) of the four Cse4-specific residues is conserved in CENP-A. In the crystal structure of the Hs HJURP/CENP-A/H4 complex, three CENP-A-specific residues (Q89, H104, and L112) in the α2 helix of CENP-A interact with three HJURP residues (F44, F29, and L18). Two of them (Q89 and H104) are in the N-terminal half region of the α2 helix (Fig. 5c). We used these two residues to substitute for the corresponding residues in Hs H3.1 and tested the binding of Hs H3.1(S88Q/G103H)/H4 to HJURP. We found that this double mutation is sufficient for recognition of the histone dimer by the HJURP chaperone in a pull-down experiment (Fig. 5d).

Discussion

Our NMR results show that the Sc and Kl Scm3/Cse4/H4 complexes have similar structures with partially unfolded histone folds for the histones, which is consistent with the high sequence homology between Sc and Kl histones and chaperones. The results confirm our earlier speculation that crystal packing along with the use of a shorter Kl Scm3 construct may have led to the canonical histone fold in the crystal structure of the Kl Scm3/Cse4/H4 complex. Indeed, Cho and Harrison have acknowledged that the region following the αN helix of Scm3 in the crystal structure of the Kl Scm3/Cse4/H4 complex is likely affected by crystal packing since it shows no interactions with the histones in the same complex. In addition, Cho and Harrison crystallized the Kl(Cse4/H4)2 tetramer.
from a solution of the Kl Scm3/Cse4/H4 complex at pH 8.5, indicating that crystal packing even con-
duces to form the (Cse4/H4)2 tetramer by dissociat-
ing Scm3 from the Cse4/Cse4/H4 complex. 12

The solution structure of the Sc Scm3/Cse4/H4 complex and the crystal structure of the Kl Scm3/Cse4/
H4 complex have different implications for structural models of the centromeric nucleosome. 23–26 For
example, the crystal structure of the Kl Scm3/Cse4/H4 complex has been used to support the hemisome
model of the Sc centromeric nucleosome since the artifactual structural regions observed for Kl Scm3 in
the Kl/Scm3/Cse4/H4 complex block DNA binding and a formation of the Kl (Cse4/H4)2 tetramer, 23–26
while the solution structure of Sc Scm3/Cse4/H4 is cited to support the octosome model. 25 More impor-
tantly, comparison of the structures of the Sc Scm3/Cse4/
H4 and Hs HJURP/CENP-A/H4 complexes now
reveals that both Scm3 and HJURP use the region
following the N-terminal helix of the chaperone to
prevent DNA from binding to the L1 loop of CenH3 and
the L2 loop of H4, even though the amino acid
sequences are not conserved.

Our studies also show that partial unfolding of
Cse4/H4 is coupled with the dissociation of the
(Cse4/H4)2 tetramer to the Cse4/H4 dimer in a pH-
and salt-dependent manner and the unfolded re-
gions of Cse4/H4 in the Scm3/Cse4/H4 complex are
also unfolded in the free Cse4/H4 dimer. In addition,
we observe conformational changes of the folded
region of Cse4/H4 in different complexes. These
results indicate that the partial unfolding in the Scm3/
Cse4/H4 complex reflects the intrinsic property of the
Cse4/H4 dimer and Scm3 binding induces confor-
mational changes in Cse4/H4. A fully folded histone
fold of Cse4/H4 may only occur in the context of other
histones and DNA when forming a nucleosome or
crystal.

The proposal that CENP-A residue S68 in the a1
helix is the primary specificity determinant for
HJURP recognition is based on pull-down exper-
iments: CENP-A with the S68Q mutation fails to
bind to HJURP, whereas Hs H3 with the inverse
Q68S mutation is recognized by HJURP. 13 The
mutation effects, however, were not observed in a
later study using chromosome-tethering assay,
biochemical reconstitution, and size-exclusion
chromatography. 17 Instead, the combination of the
two C-terminal substitutions (H104 and L112)
in the a2 helix of CENP-A with either one of the
two substitutions in the N-terminal portion of the a2
helix (Q89) or the L1 loop (N85) is sufficient to
target CENP-A to the chromosomal HJURP array.
Our mutation studies reveal that the N-terminal
region of the a2 helix of CenH3, which includes
either four Cse4-specific residues or two CENP-A-
specific residues, is sufficient for chaperone rec-
nognition. Thus, while the sequence and overall
structures of the CenH3/chaperone complexes
have changed significantly throughout evolution,
the structural region for chaperone recognition has
largely been maintained.

In summary, Cse4/H4 in the budding yeast Scm3/
Cse4/H4 complex has a partially unfolded structure,
reflecting the intrinsic property of the Cse4/H4 dimer.
Partial unfolding in the Cse4/H4 dimer is coupled
with the dissociation of the (Cse4/H4)2 tetramer.
Scm3 associates with the partially unfolded Cse4/H4
dimer and induces structural changes in the his-
tones. Both Scm3 and HJURP use the region
following the aN helix to block the DNA-binding
sites in CenH3/H4. The N-terminal portion of the
CenH3 a2 helix contains the conserved chaperone
recognition region. Our results provide an example
of how protein complexes can evolve their se-
quences and overall structures while maintaining
local regions for specific recognition.

Materials and Methods

Gene cloning of histones and chaperones

For efficient expression and purification of histones and
chaperones, the codon-optimized DNAs encoding a single
chain of His6-Cse4 106–183-LVPRGS-H4 103–106, and His6-
SSGLVPRGS-CENP-A 60–140-LVPRGS-HJURP 143–183 were
synthesized commercially (GeneWiz, USA) and cloned into
a pET vector (Novagen, USA). The LVPRGS is the
sequence recognized by thrombin, which cuts between R
and G. The mutant construct His6-Cse4 106–183(L158G/
L159G/L161G/H162G)-LVPRGS-H4 103–106 was derived
from the above construct by site-directed mutagenesis
using the QuikChange kit (Stratagene, USA). Also, the
DNAs for His6-SSGLVPRGS-Csm3 61–143 and H4 103–106
were synthesized and cloned into a pET vector, respectively.
The DNAs encoding H3 61–136 and H3 61–136(S88M/
G91M/S96A/V97S) were amplified from the plasmids
containing wild-type H3 61–136 (with amino acid sequence
identical to H3 61–136) or mutant H3 61–136(S88M/G91M/S96A/
V97S) 11 and sub cloned into a pET vector, respectively.
The cDNA encoding full-length H3.1 was amplified from
SuperScript human brain cDNA library (Life Technologies,
USA) and cloned into a pET vector. Full-length human H4
was a gift from Dr. Carl Wu. Human H4 1–151-LVPRGS-
H4 103–136 and full-length H3.1(S88Q/G103H) were pro-
duced by site-directed mutagenesis from the full-length
human H4 and H3.1 constructs using the QuikChange kit
(Stratagene). The plasmid encoding a single-chain His6-
KK-Cse4 5c 151–207-LVPRGS-H4 103–145 was described in
our early work. 11 A list of constructs used in this study
is summarized in Table S1.

Expression and purification of recombinant proteins

Escherichia coli strain BL21-CodonPlus(DE3)-RIPL (Strata-
gene) was used for the expression of histone and chaperone
proteins. The purification of H3 61–136, H3 61–136(S88M/
G91M/S96A/V97S), H4 1–103, H4 1–103, and H4 1–103
was described in our early work. 11 A list of constructs used in this study
is summarized in Table S1.
followed the published procedure. The His₆-tagged proteins were purified as described in our earlier work. Isotope-labeled proteins for NMR studies were expressed by growing *E. coli* cells in M9 media with ¹⁵NH₄Cl, with U-¹³C₆-glucose, and with or without D₂O as the sole source for nitrogen, carbon, and deuterium, respectively.

**Reconstitution of protein complexes**

To prepare the Scm₃/Kl/Cse₄/Kl/H₄/Kl complex, we mixed lyophilized Histagged Scm₃⁴₃–143 and His₆-Cse₄¹₀₆–183-LVPGRS-H₄²⁸₀–₃₀₃ complexes in 6 M GuHCl and then dialyzed it overnight against a buffer containing 10 mM Tris–HCl and 0.5 M NaCl (pH 7.4) at 4 °C. Trichloroacetic acid was then added to the solution to cleave the linker. The proteins were denatured again in 6 M GuHCl and dialyzed overnight against the same buffer at 4 °C. The soluble fraction was concentrated and subjected to gel filtration on a HiLoad 16/60 Superdex 200 column (GE Healthcare, USA). The eluted complex was concentrated and buffer exchanged with 50 mM Mes (pH 5.6). The GS-Scm₃ complex was concentrated and buffer exchanged with 50 mM Mes (pH 5.6) and 150 mM NaCl.

**Analytical ultracentrifugation experiments**

A complex containing GS-Scm₃⁴₃–143, His₆-Cse₄¹₀₆–183(L158G/L159G/L161G/H162G)-LVPR, and GS-H₄²⁸₀–₃₀₃ was studied in 50 mM Mes (pH 5.6) at a concentration corresponding to a measured A₄⁸₅ of 0.80 (35 μM). In addition, samples containing His₆-Cse₄¹₀₆–183-LVPGRS-H₄²⁸₀–₃₀₃ and GS-H₄²⁸₀–₃₀₃ were studied at a dimer concentration of 38 μM in different buffers, including 50 mM Mes (pH 5.6), 50 mM Mes (pH 5.6) and 150 mM NaCl, 50 mM phosphate (pH 6.5) and 150 mM NaCl, and 50 mM phosphate (pH 7.4) and 150 mM NaCl.

**Sedimentation velocity experiments**

Sedimentation velocity experiments were conducted at 20 °C on a Beckman Optima XL-A or Beckman Coulter ProteomeLab XL-I analytical ultracentrifuge. Four hundred microliters of each of the samples was loaded in two-channel centerpiece cells and analyzed at a rotor speed of 50 krp/min with scans collected at approximately 5-min intervals. Data were collected using the absorbance optical system in continuous mode at 280 nm using a radial step size of 0.003 cm. Data were also collected using the Rayleigh interference detection system when possible. All data were analyzed in SEDFIT 12.7 (in terms of a continuous c(s) distribution of Lamm equation solutions covering a maximal s₁₀₀,₀ range of 0–10 with a resolution of 20 points per s and a confidence level of 0.68). Excellent fits were obtained with RMSD values ranging from 0.0034 to 0.0047 absorbance units. Solution densities ρ and viscosities η were measured as previously described or calculated in SEDNTERP 1.09. Partial specific volumes v were calculated in SEDNTERP 1.09 and corrected to account for the ¹⁵N, ¹³C, and/or ²H isotopic substitution. Sedimentation coefficients s were corrected to s₁₀₀,₀.

**NMR experiments and structure calculation**

NMR experiments were performed on Bruker 700- and 900-MHz spectrometers at 30 °C (Kl/Scm₃/Cse₄/H₄) or 35 °C (Sc Cse₄-H₄). The following experiments were recorded: 2D [¹H, ¹⁵N]-HSQC, [¹H, ¹⁵N]-TROSY (transverse relaxation optimized spectroscopy); 3D CBCANH, CBCA(CO)NH, HNCO, HN(CA)CO, HCNA, HN(CO)CA, HBCANH, HOCCH-TOSY (total correlated spectroscopy), CCH-TOSY, CCH(CO)NH, [¹H, ¹³C]-NOESY, [¹H, ¹³C]-NOE, [¹H, ¹³C]-NOESY-HSQC, TROSY version 3D, HNACB, HN(CO)CA, HNCO, HN(CA)CO. The spectra were processed using NMRPipe and analyzed with Sparky (Godard and Kneller). Structure calculation was done as described in our earlier work. The program PROCHECK-NMR was used to evaluate the quality of the calculated structures.

**Pull-down experiments**

Pull-down experiments were carried out in 20 mM Tris–HCl (pH 8.0), 50 mM imidazole, 2 M NaCl, and 5 mM MES at 4 °C. Five micromolar His₆-H₃ (Kl/His₆/H₄₁–₁₃₆/H₄₁–₁₃₆ or His H₃₁,₁–₁₃₆-His₆/H₄₁–₁₃₆) or H3 mutant/H₄ (Kl/His₆/H₄₁–₁₃₆, S88G/M91/S96A/V97S)/H₄₁–₁₃₆ or His H₃₁,₁–₁₃₆ (S88G/G103H-His₆/H₄₁–₁₃₆) with corresponding equal molar amount of histone chaperone K/His₆-SGLVPGRS₃₃₄–₁₄₃ or His GS-HJURP₂–₈₁ were incubated with Ni-NTA (Qiagen) beads for 1 h, washed 5 times, eluted from the beads with 500 mM imidazole, and then analyzed by SDS-PAGE.
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Supplementary Data

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Hs, Homo sapiens; Sc, Saccharomyces cerevisiae; Kl, Kluyveromyces lactis; HSQC, heteronuclear single quantum correlation; Mes, 4-morpholineethanesulfonic acid; βMEß-mercaptopethanolTROSY, transverse relaxation optimized spectroscopy.

References


