

Crystal Structure of the H3.3 Histone Chaperone Complex Subunit HIRA and its Functional Role

The HIRA complex, which comprises HIRA, UBN1, and CABIN1, is a histone chaperone for histone variant H3.3, mediating the incorporation of H3.3 into nucleosomes at transcribed regions and DNA repair sites. We found that HIRA acts as a trimer, as revealed by X-ray crystallographic and biochemical analyses. HIRA trimerization is essential for its localization at UV damage sites, H3.3 deposition, and interaction with its partner protein, CABIN1. The homotrimeric HIRA may serve as a platform interacting with components of transcription or repair machinery present at DNA bubble structures.

Eukaryotic chromatin consists of a repeat of nucleosomes, in which DNA is wrapped around a protein complex called a histone octamer. The histone octamer contains two molecules of each core histone, H2A, H2B, H3, and H4. Inheritance, expression, and repair of eukaryotic genetic materials usually involve chromatin remodeling, including nucleosome assembly and/or disassembly. The nucleosome assembly/disassembly is facilitated by a group of proteins called histone chaperones. Histone chaperones are associated with histones and stimulate a reaction involving histone transfer without being a part of the final product. The histone regulator A (HIRA) is a histone chaperone for the histone variant H3.3, mediating its incorporation to nucleosomes at transcribed regions and DNA repair sites [1, 2]. HIRA forms a complex with Calcineurin-binding protein 1 (CABIN1) and Ubinuclein 1 (UBN1) [3]. In order to reveal a functional oligomerization state of the HIRA complex, we conducted biochemical and crystallographic analyses [4].

After expression by *E. coli*, the C-terminal region of human HIRA (aa 644-1017) was purified in 300 mM NaCl (Fig. 1a). However, the purified protein was precipitated during concentration, and it could not be concentrated to higher than 1 mg/ml. Analysis of size-

exclusion chromatography with in-line multi-angle light scattering (SEC-MALS) detected an unstable refractive index (Fig. 1b). This problem was solved by increasing the NaCl concentration to 500 mM. Under this condition, HIRA(644-1017) could be concentrated to more than 10 mg/ml (Fig. 1c). SEC-MALS analysis indicated that the molecular weight of HIRA(644-1017) was $111,400 \pm 2.3\%$, which was comparable to the theoretical molecular weight of a trimer, 125,815 (Fig. 1d).

The purified HIRA(644-1017) (Fig. 1c) was crystallized using an automated protein crystallization and monitoring system, PXS [5]. The initial crystallization screening gave crystals from no. 14 of the Stura Footprint (Molecular Dimensions). The crystallization condition was further optimized based on the above condition by changing the concentration of the precipitant and pH. Moreover, Additive Screen (Hampton Research) and microseeding were used for the optimization. Finally, we obtained single crystals of HIRA(644-1017). Diffraction data for Se-SAD (single wavelength anomalous diffraction) phasing was collected with an X-ray of wavelength 0.9790 Å at beamline BL41XU of SPring-8 (Hyogo, Japan). Native-SAD data was collected with an X-ray of wavelength 1.9000 Å at beamline BL-17A at Photon Factory (KEK, Ibaraki, Japan). While initial

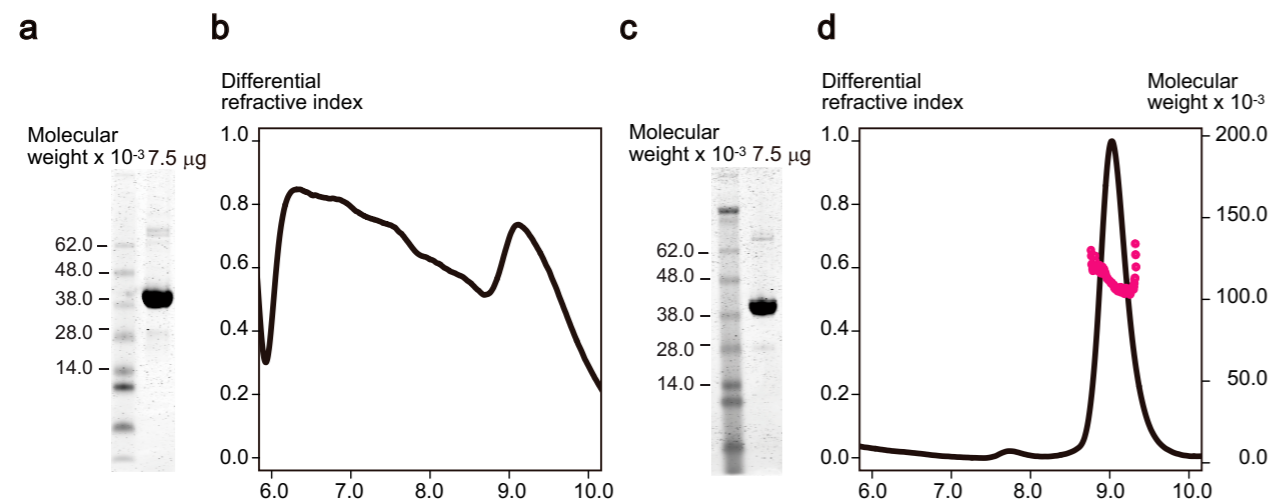


Figure 1: SEC-MALS analysis of HIRA(644-1017). **a** SDS-PAGE gel for HIRA(644-1017) in 300 mM NaCl and **b** SEC-MALS analysis of HIRA(644-1017) in 300 mM NaCl. **c** SDS-PAGE gel for HIRA(644-1017) in 500 mM NaCl and **d** SEC-MALS analysis of HIRA(644-1017) in 500 mM NaCl. The line indicates differential refractive index (left axis) and the dots indicate molecular weight (right axis).

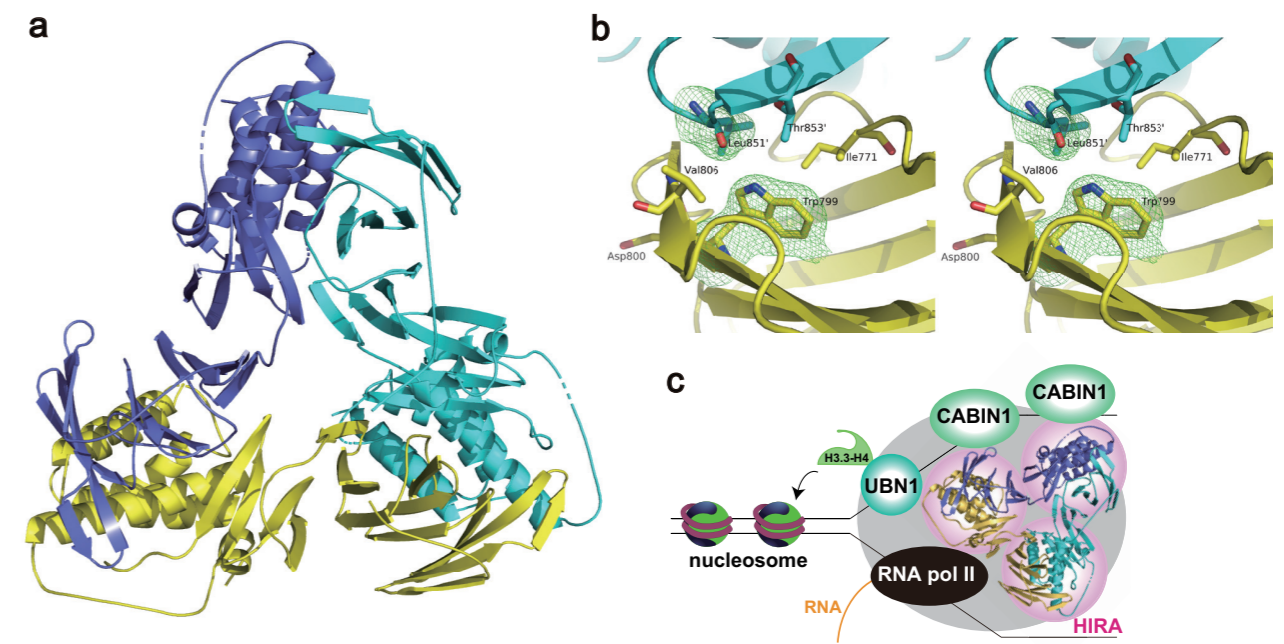


Figure 2: Crystal structure of HIRA(644-1017). **a** Overall structure of homotrimeric HIRA(644-1017). Subunits A, B, and C are shown in yellow, cyan and blue, respectively. **b** Trp799 located at the interface between subunits A and B, shown in the stereo image. The mFo-DFc simulated annealing (SA)-omit map for Trp799 in subunit A and Leu851 in subunit B is shown in green. The density map was counteracted at 3σ . **c** Model for HIRA homotrimer function.

phases of the HIRA(644-1017) were obtained by the Se-SAD method, it was not possible to build the model for the C-terminal region of HIRA(644-1017) due to poor electron density. Accordingly, we obtained the molecular replacement (MR)-native SAD electron density map. The anomalous difference Fourier map gave coordinates of sulfur atoms in not only Met but also Cys residues, which helped us to construct the HIRA(644-1017) model. The crystal structure of HIRA(644-1017) was crystallographically refined at 2.45 Å resolution, of which diffraction data was collected with an X-ray of wavelength 0.9800 Å at beamline BL-17A (PF). The asymmetric unit of the crystal contained a homotrimer of HIRA(644-1017) (Fig. 2a). SEC-MALS analysis also showed that HIRA(644-1017) forms a trimer in solution (Fig. 1d). Immunoprecipitation analysis using YFP-tagged HIRA(492-1017) and YFP-tagged HIRA(1-440) revealed that endogenous HIRA co-immunoprecipitated with YFP-tagged HIRA(492-1017) but not with YFP-tagged HIRA(1-440), suggesting that HIRA(644-1017) forms an oligomer (probably a trimer) in cells. Immunoprecipitation analysis demonstrated that point mutations in Trp799 and Asp800 hampered homo-oligomerization of HIRA. While Asp800 does not interact with an adjacent subunit in the crystal structure of HIRA(644-1017), Trp799 is located at the interface of two subunits and forms a hydrophobic patch with Ile771 and Val806. Since this hydrophobic patch interacts with Leu851 and Thr853 of an adjacent subunit (Fig. 2b), substitution of Trp799 seems to reduce the hydrophobic interaction between the subunits. Further cell biological experiments

confirmed that HIRA homotrimerization is essential for its localization at UV damage sites, H3.3 deposition and interaction with CABIN1. Sedimentation analysis with CABIN1 revealed that the HIRA (aa 661-1017)-CABIN1 complex contains three HIRA and two CABIN1 molecules. These observations strongly suggest that the functional oligomeric state of HIRA is a homotrimer. We speculate that homotrimeric HIRA acts as a platform for multiple players of transcription or repair machineries present at DNA bubble structures (Fig. 2c).

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BEAMLINES

BL-1A, BL-5A, BL-17A and AR-NE3A

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