Structural Basis for Site-Specific Nucleosome Disassembly by Histone Chaperone CIA/ASF1 Complexed with a Double Bromodomain

Site-specific nucleosome disassembly and reassembly are essential for transcription, DNA replication, and DNA repair. Although histone acetylation marks the transcriptional start-site of active genes and induces nucleosome disassembly in a site-specific manner, the molecular mechanism underlying site-specific nucleosome disassembly remains elusive. We determined the crystal structure of histone chaperone CIA/ASF1 in complex with a double bromodomain, an acetylated histone-recognizing domain, at 3.3 Å resolution. Our crystallographic and biological analyses suggested that CIA/ASF1 is recruited to a promoter region of active genes through interaction with DBD (CCG1) and transferred to histone H3–H4 for the site-specific nucleosome disassembly.

The eukaryotic genome is composed of nucleosomes, which are complexes of DNA and histones. Since enzyme access to DNA is hampered by tight interaction between DNA and histones, the nucleosome should be site-specifically disassembled for nuclear reactions such as transcription, DNA replication, and DNA repair. Although histone acetylation marks the transcription start site of active genes and induces site-specific nucleosome disassembly, the molecular mechanism underlying site-specific nucleosome disassembly remains elusive. We focused on the functional interaction between histone chaperone CIA/ASF1, which has histone $(H3–H4)_2$-disrupting activity [1], and an acetylated histone-recognizing double bromodomain [DBD (CCG1)] in general transcription factor TFIID. This interaction seems to link histone acetylation to the site-specific nucleosome disassembly [2]. Here, we report the crystal structure of the CIA/ASF1–DBD (CCG1) complex and its functional significance.

CIA/ASF1 and DBD (CCG1) were expressed in *Escherichia coli* and purified. The CIA/ASF1–DBD (CCG1) complex was crystallized by the hanging-drop vapor diffusion method at 293 K. Crystals with dimensions of 0.4 × 0.25 × 0.25 mm^3 were obtained and belonged to the space group $P6_122$ with unit cell parameters of $a = b = 102.12$ Å and $c = 271.92$ Å. The X-ray diffraction data were collected at AR-NW12A. The crystal structure of the CIA/ASF1–DBD (CCG1) complex was determined at 3.3 Å resolution by the molecular replacement method with search models of 1ROC (CIA/ASF1) and 1EQF [DBD (CCG1)].

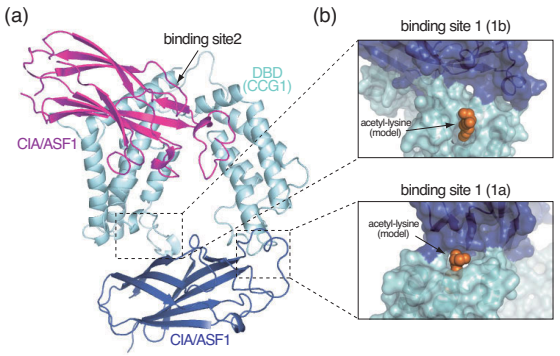

Figure 1
Overall structure of CIA/ASF1–DBD(CCG1) complex.
(a) Crystal structure of CIA/ASF1–DBD(CCG1) complex. (b) The acetylated histone binding sites of DBD(CCG1) (cyan) with CIA/ASF1 (deep blue) and modeled acetylated lysine residues (orange). The model structures of acetylated lysine residues were prepared on the basis of the crystal structure of the Gcn5p bromodomain in complex with the acetylated histone H4 peptide [4].

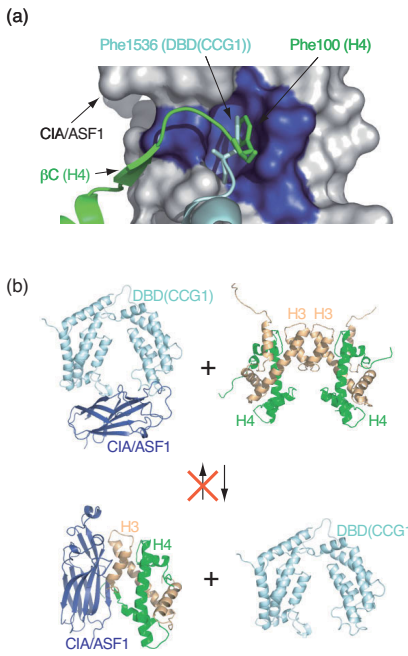

Figure 2
Interacting surface among CIA/ASF1, DBD(CCG1), and histone H3–H4.
(a) Interacting surface of DBD(CCG1) and histone H4 for CIA/ASF1 at binding site 1. DBD(CCG1), histone H4, and CIA/ASF1 are shown in cyan, green, and gray, respectively. The hydrophobic pocket of CIA/ASF1 is colored deep blue. Phe1536 and Phe100 for DBD(CCG1) and histone H4 are shown by a stick model. (b) Summary of *in vitro* competition assay. DBD(CCG1) was dissociated from CIA/ASF1 dose-dependently by adding the histone $(H3–H4)_2$ to the CIA/ASF1–DBD(CCG1) complex.

The crystal structure showed that one DBD (CCG1) molecule binds two CIA/ASF1 molecules in two distinct sites [Fig. 1(a)]. The small interacting surface areas of the two sites suggested weak interactions between CIA/ASF1 and DBD (CCG1). On the basis of the crystal structure, the interaction between CIA/ASF1 and DBD (CCG1) was analyzed in solution by a GST pull-down assay, isothermal titration calorimetry, and analytical ultracentrifugation. The results showed that DBD (CCG1) has binding sites 1 and 2 for CIA/ASF1 in solution with *K*d values of 8.6 μM and 173 μM, respectively.

The crystal structure of the CIA/ASF1–DBD (CCG1) complex seems to explain the link between histone acetylation and site-specific histone eviction. First, since the acetylated histone-binding sites of DBD (CCG1) are exposed to the solvent in the complex [Fig. 1(b)], the complex seems to interact with acetylated histone tails. Indeed, an acetylated-histone H4 peptide interacts with the CIA/ASF1–DBD (CCG1) complex, suggesting that CIA/ASF1 could be recruited to the targeted promoter site through interaction with DBD (CCG1). Second, the superposition of CIA/ASF1 in the CIA/ASF1–H3–H4 complex [1] onto CIA/ASF1 (at binding site 1) in the CIA/ASF1–DBD (CCG1) complex showed that DBD (CCG1) and histone H3–H4 compete for CIA/ASF1 [Fig. 2(a)]. The *in vitro* competition assay showed that CIA/ASF1 is transferred from DBD (CCG1) to histone H3–H4 [Fig. 2(b)]. We also analyzed the functional significance of the CIA/ASF1–DBD (CCG1) interaction *in vivo*. The chromatin immunoprecipitation analyses suggested that the interaction between CIA/ASF1 and DBD (CCG1) at binding site 1 is involved in CIA/ASF1 recruitment, histone eviction, and RNA polymerase II entry around the active promoter region.

These structure-based biochemical and biological analyses suggested a molecular mechanism by which CIA/ASF1 is recruited to a promoter region of active genes through the interaction with DBD (CCG1), and that the recruited CIA/ASF1 changes its interacting partner from DBD (CCG1) to histone H3–H4, leading to the site-specific nucleosome disassembly (hi-MOST model) [3]. The hi-MOST model is the first molecular mechanism to explain the site-specific nucleosome disassembly.

REFERENCES

[1] R. Natsume, M. Eitoku, Y. Akai, N. Sano, M. Horikoshi and T. Senda, *Nature* **446** (2007) 338.
[2] T. Chimura, T. Kuzuhara and M. Horikoshi, *Proc. Natl. Acad. Sci. USA* **99** (2002) 9334.
[3] Y. Akai, N. Adachi, Y. Hayashi, M. Eitoku, N. Sano, R. Natsume, N. Kudo, M. Tanokura, T. Senda and M. Horikoshi, *Proc. Natl. Acad. Sci. USA* **107** (2010) 8153.
[4] D.J. Owen, P. Ornaghi, J.C. Yang, N. Lowe, P.R. Evans, P. Ballario, D. Neuhaus, P. Filetici and A.A. Travers, *EMBO* **19** (2000) 6141.

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