

## Crystal structure of the histone chaperone CIA–histone-H3–H4 complex

Ryo NATSUME<sup>1</sup>, Yusuke AKAI<sup>1</sup>, Masami Horikoshi<sup>2</sup>, Toshiya SENDA\*<sup>3</sup>

<sup>1</sup>Res. & Dev. Dept., JBIC, Aomi 2-42, Koto-ku, Tokyo 135-0064, Japan

<sup>2</sup>IMCB, The Univ. of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-0032, Japan

<sup>3</sup>BIRC, AIST, Aomi 2-42, Koto-ku, Tokyo 135-0064, Japan

### Introduction

In eukaryotes, genomic DNA and histone proteins form the basic structural unit of chromatin the nucleosome. In the nucleosome, DNA is wrapped around a histone octamer that is composed of a histone (H3–H4)<sub>2</sub> tetramer and two flanking histone H2A–H2B dimers. Since the nucleosome structure sterically inhibits interactions between protein factors and DNA, it must be disassembled to allow protein factors to promote reactions on the DNA. Thus, the histone chaperones that mediate nucleosome assembly and disassembly serve a key role in the regulation of transcription, replication, and DNA repair.

CIA is the most evolutionarily conserved histone chaperone. It is involved in transcription, replication, and DNA repair. CIA preferentially interacts with histones H3 and H4, and facilitates both nucleosome assembly and disassembly. In order to elucidate the molecular mechanism of nucleosome assembly and disassembly by CIA, we determined the crystal structure of CIA in complex with histones H3 and H4[1]. Here, we report the structural and biochemical analyses of CIA.

### Methods

The complex was prepared by mixing CIA and histone (H3–H4)<sub>2</sub> tetramer at a 1:2 molar ratio. Although these proteins immediately formed precipitates upon mixing, they could be dissolved by increasing the NaCl concentration to 0.76 M. Crystallization screening was performed by the hanging-drop vapour diffusion method using Crystal Screen I & II (Hampton Research). Crystals that diffracted beyond 3.0 Å resolution using synchrotron radiation were obtained from the reservoir condition of Crystal Screen II reagent #34.

Diffraction data were collected at BL-NW12A. The obtained data were processed and scaled with MOSFLM and SCALA, respectively. The crystal structure of the CIA–histone-H3–H4 complex was solved using the molecular replacement method with MOLREP. The molecular model was built with XtalView and refined with CNS and REFMAC5 at 2.7 Å resolution ( $R_{\text{work}}=0.238$ ,  $R_{\text{free}}=0.293$ ).

### Results and Discussion

In the crystal, CIA binds a histone H3–H4 dimer (Figure 1a). The crystal structure shows that the H3–H4 dimer interacts with CIA utilizing two surfaces: (i) helix  $\alpha 3$  and the C-terminal half of helix  $\alpha 2$  of histone H3 and

(ii) the C-terminal  $\beta$ -strand ( $\beta c$ ) of H4. Helix  $\alpha 3$  and the C-terminal half of helix  $\alpha 2$  of histone H3 are also utilized for the histone H3–H3 interaction in the histone (H3–H4)<sub>2</sub> tetramer (Figure 1b). Therefore, the interaction between CIA and the histone H3–H4 dimer inhibits the formation of a histone (H3–H4)<sub>2</sub> tetramer. Since the CIA–histone-H3–H4 complex was crystallized from a solution containing CIA and the histone (H3–H4)<sub>2</sub> tetramer, CIA seems to have histone (H3–H4)<sub>2</sub> tetramer-splitting activity. The molecular-weight analysis using the static-light scattering method clearly showed that CIA has the ability to split the histone (H3–H4)<sub>2</sub> tetramer into two histone H3–H4 dimers through forming CIA–histone-H3–H4 complexes. Since many researchers have assumed that the histone (H3–H4)<sub>2</sub> tetramer is a stable complex in solution, the present result should have a great impact on chromatin research. This is the first experimental evidence showing that an endogenous factor can split a histone (H3–H4)<sub>2</sub> tetramer into two histone H3–H4 dimers.

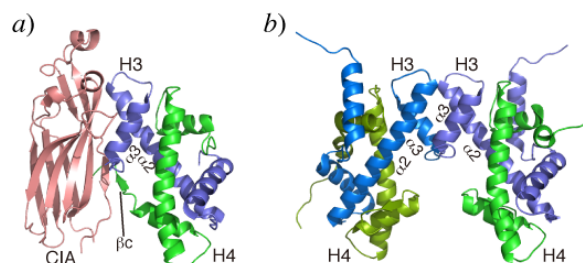


Figure 1. **a**) Crystal structure of the CIA–histone-H3–H4 complex. **b**) The structure of a histone (H3–H4)<sub>2</sub> tetramer in the crystal structure of a nucleosome.

In addition, our comprehensive *in vivo* mutational analysis of CIA using budding yeast showed that the mutation on residues involving the interaction between CIA and histone H3 caused the *SPT(-)* phenotype. This result suggested that the interaction between CIA and histone H3–H4 dimer is important for transcriptional reaction. Taken together, we can tentatively conclude that the CIA–histone-H3–H4 complex occurs as an intermediate in the nucleosome assembly and disassembly processes during transcription.

### Reference

[1] R. Natsume et al., Nature. 446, 338 (2007).

\* toshiya-senda@aist.go.jp