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X-ray crystallographic analysis of the (Runx1–CBFβ)$_2$–Ets1–DNA complex assembled on the enhancer of T cell receptor α chain gene

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Introduction

Gene-specific transcription is involved in almost every cellular function, and an understanding of its regulation mechanism will give impact on the large area of biological researches. Gene-specific transcription is initially regulated through assembly of transcription factor (TF)–DNA complex formed on gene enhancers. To understand the mechanism by which TF–DNA-complex formation is regulated, we studied the T cell-specific TF–DNA complex formed on the *T cell antigen receptor (TCR) α chain* enhancer. This complex is known to contain multiple TFs such as Runx1, Ets1, CBFβ, CREB, etc., which synergistically bind to the *TCRα* enhancer, activating the target gene. Although each TF has been individually studied well, it remains unclear how they behave in the complex at the structural level. For example, a component of the complex, Ets1, is individually studied well: Ets1 activity is negatively regulated by the regulatory region (the exon VII) flanking its DNA binding domain (so called the ETS domain) and phosphorylations of the exon VII suppress a DNA binding activity of Ets1 more significantly. However, how Ets1 would behave in TF–DNA complexes are almost unknown. Here, we performed crystallographic analysis for the (Runx1–CBFβ)$_2$–Ets1–DNA complex.

Experiment

Bacterially expressed TFs, Ets1, Runx1 and CBFβ were purified and mixed with various lengths of double-stranded DNA containing *TCRα* enhancer in the following molar ratio: Runx1/CBFβ/Ets1/DNA = 2/2/1/1. The complex formation was checked by electrophoretic mobility shift assay and SDS-polyacrylamide gel electrophoresis. Then the complex solution was subjected to initial crystallization screening using Natrix, a crystallization reagents kit for nucleic acids (Hampton Research). The (Runx1–CBFβ)$_2$–Ets1–DNA complex was crystallized and soaked in cryoprotectant solutions, followed by flush-cooling in a stream of cold nitrogen gas at 100 K. Diffraction images were collected with synchrotron radiation at NW12A and BL-17A in Photon Factory and processed using HKL2000.

Results and Discussion

The crystals belong to the monoclinic space group *C*2, with unit cell dimensions of a = 330.0, b = 89.4 and c = 146.7. Due to a long a-axis, we had to reduce the angular width of the oscillation. The crystals were relatively resistant to a damage by X-rays and diffracted to 3.8 Å. Selection of the cryoprotectant agents was critical for diffraction quality.

We are currently trying to improve resolution and diffraction quality of the crystals. In parallel, we perform functional analyses of this complex using site-directed mutagenesis based on the obtained preliminary crystal structure.

Table 1: Data-collection statistics of (Runx1–CBFβ)$_2$–Ets1–DNA complex

Beam-line	BL17A
Wavelength	0.98
Resolution (Å)	50.0-3.8
Space group	*C*2
Unit-cell parameters (Å)	
a	330.0
b	89.4
c	146.7
β	115.7°
No. of reflections	
Observed	138731
Unique	37854
Completeness (%)	99.6 (97.1)[#]
I/σ (I)	12.5 (1.8)[#]
R_{merge} (%)	11.4

[#] Numbers in parentheses refer to data for high resolution outer shell 3.87-3.80 Å

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