BL-1A, 5A, 17A/2011G600

# X-ray crystallographic analysis of the LEF1-Runx1-CBFβ -DNA complex assembled on the enhancer of T cell receptor α chain gene

Masaaki SHIINA, Keisuke HAMADA, Taiko BUNGO, Mariko SHIMAMURA, Shiho BABA, Kazuhiro OGATA*
Dept. of Biochemistry, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

## 1 Introduction

Gene-specific transcription is involved in many cellular functions, 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 have been studying 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 LEF1, Runx1, Ets1, CBFβ, CREB, etc., which synergistically bind to the *TCRα* enhancer, activating gene transcription. We have engaged in structural and functional analyses of the higher-order TF-DNA complex formed on the *TCRα* enhancer. We have already solved high and low resolution structures of the Ets1-Runx1-CBFβ-DNA and $(Runx1\text{-}CBF\beta)_2$-Ets1-DNA complexes, respectively, and now have started crystallographic analyses of the LEF1-Runx1-CBFβ-DNA complex formed on the *TCRα* enhancer.

## 2 Experiment

Bacterially expressed TFs, LEF1, Runx1 and CBFβ, were purified and mixed with double-stranded DNA containing *TCRα* enhancer sequence in an equal molar ratio. The complex formation was checked by electrophoretic mobility shift assay (EMSA) 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). By modifying crystallization conditions and procedures, we obtained crystals of LEF1-Runx1-CBFβ-DNA complex, and the obtained crystals were 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 BL-5A and BL-17A in Photon Factory and were processed using HKL2000.

## 3 Results and Discussion

The crystals belong to the trigonal space group $P3_1$ or $P3_2$, with unit cell dimensions of $a = b = 92.8$ Å, $c = 75.3$ Å. The crystals were relatively resistant to a damage by X-rays and diffracted to 3.1 Å. The structure of the complex showed that a DNA molecule is kinked by the HMG domain of LEF1 and forms pseudo-continuous helix with overhanging ends paired. Although a weak but clear presence of cooperative DNA binding between LEF1 and Runx1 was shown by EMSA, we could not observe inter-molecular interaction between them in the crystal structure of the complex at the current resolution. Accordingly, we are trying to improve resolution and diffraction quality of the crystals. In parallel, we are performing functional analyses of this complex using site-directed mutagenesis based on the crystal structure.

Table 1: Data-collection statistics of LEF1-Runx1-CBFβ-DNA complex

| | |
|---|---|
| Beam-line | BL-5A |
| Wavelength | 1.00 |
| Resolution (Å) | 50.0-3.1 |
| Space group | $P3_1$ or $P3_2$ |
| Unit-cell parameters (Å) | |
| *a* | 92.8 |
| *b* | 92.8 |
| *c* | 75.3 |
| *α, β, γ* | 90°, 90°, 120° |
| No. of reflections | |
| Observed | 73881 |
| Unique | 12791 |
| Completeness (%) | 100 (97.7)[#] |
| $I/\sigma$ (*I*) | 25.7 (4.4)[#] |
| $R_{merge}$ (%) | 6.0 (45.1) |

[#] Numbers in parentheses refer to data for high resolution outer shell 3.15-3.10 Å

* ogata@med.yokohama-cu.ac.jp