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X-ray crystallographic analysis of the $(Runx1)_2$-DNA complex assembled on the enhancer of T cell receptor α chain gene; a possible role of the intrinsically disordered region of Runx1

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1 Introduction

Gene-specific transcription is initially regulated through assembly of transcription factors (TFs)-DNA complex formed on an enhancer.

To reveal the mechanism of the TF-DNA assembly, we have been studying the T cell-specific assembly formed on the *T cell antigen receptor* (*TCR) α chain* enhancer. This complex is comprised of multiple TFs including two Runx1-CBFβ heterodimers, LEF1, Ets1, and CREB, which bind cooperatively to the *TCRα* enhancer. In this study, we solved the crystal structure of the $(Runx1)_2$-DNA complex formed on the *TCRα* enhancer, using the Runx1 fragment containing its N-terminal intrinsically disordered region (IDR). We found that the IDR of Runx1 regulates cooperative DNA binding between two Runx1 molecules.

2 Experiment

Bacterially expressed Runx1 fragment containing the N-terminal IDR was purified and mixed with double-stranded DNA having *TCRα* enhancer sequence, which includes two binding sites of Runx1, in the two to one molar ratio of protein and DNA. 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 reagent kit for nucleic acids (Hampton Research). By modifying crystallization conditions and procedures, we obtained crystals of the $(Runx1)_2$-DNA complex. Then 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 space group *P222*, with unit cell dimensions of a = 87.0 Å, b = 88.0 Å, c = 97.6 Å. The crystals were relatively resistant to damage by X-rays and diffracted to 2.2 Å (Table 1). The structure was solved by molecular replacement with a published Runx1 structure. The obtained structure showed that the two Runx1 molecules bind to the *TCRα* enhancer DNA in the head-to-tail orientation. The electron densities corresponding to the N-terminal IDRs of the bound Runx1 molecule were clearly observed in one of the two Runx1 molecules. This suggests that the N-terminal IDR of one of the two Runx1 molecules becomes folded by cooperative binding of them to the *TCRα* enhancer. We are now exploring biological implication of the induced folding of the N-terminal IDR of Runx1 using site-directed mutagenesis based on the crystal structure.

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

Beam-line	BL-5A
Wavelength	1.00
Resolution (Å)	50.0-2.2
Space group	*P222*
Unit-cell parameters (Å)	
a	87.0
b	88.0
c	97.6
α, β, γ	90°, 90°, 90°
No. of reflections	
Observed	415812
Unique	38543
Completeness (%)	99 (100)[#]
I/σ (*I*)	56.6 (8.0)[#]
R_{merge} (%)	6.9 (49.1)

[#] Numbers in parentheses refer to data for high resolution outer shell 2.24-2.28 Å

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