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

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Introduction

Target gene specificity in transcriptional regulation is determined by an enhanceosome comprised of a distinct set of multiple transcription factors formed on a *cis*-element in an enhancer region. An enhanceosome is assumed to respond dynamically to various cell signals through chemical modifications, such as phosphorylation, of transcription factors. Phosphorylation of transcription factors is reported to control transactivation of target genes. However, the role of phosphorylation of transcription factors in the context of an enhanceosome on a target gene enhancer remains unclear. To reveal the underlying mechanism, we focused on the Ets1–Runx1–CBFβ–DNA complex, the most important part of the enhanceosome formed on the *TCR*α enhancer. 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 more strongly DNA binding activity of Ets1. Runx1, which binds to *TCR*α enhancer with Ets1 side by side, counteracts the self-inhibition of DNA binding activity of Ets1, leading to the cooperative DNA binding. To elucidate the mechanism of enhanceosome formation, we performed crystallographic analyses of complexes composed of Ets1, Runx1, CBFβ and DNA derived from the *TCR*α gene enhancer.

Experimental Procedures

Diffraction data were collected on beamlines BL17A and NW12A at the Photon Factory at KEK (Tsukuba, Japan). The structures of the complexes comprised of Ets1, Runx1, CBFβ, and DNA were solved by the molecular replacement method using the program Phaser, with the structure of the Runx1–CBFβ–DNA complex (PDB entry 1IO4) (Tahirov et al., 2001) as a search model. Refinement and model building were performed using the programs CNS and Coot, respectively.

Results and Discussion

It has been suggested that direct protein–protein interactions between Ets1 and Runx1 are implicated in their functional cooperativity. However, there was no Ets1–Runx1 interaction in our crystal structures, even though the Ets1 and Runx1 fragments used for crystallization, Ets1(276–441) and Runx1(60–263), include the regions that are reported to be involved in the interactions between Ets1 and Runx1 (Figures 1). The structures of the Runx1 and CBFβ elements in the Ets1–Runx1–CBFβ–DNA complex are similar to those of the Runx1–CBFβ–C/EBPβ–DNA complex we previously reported (PDB ID: 1IO4) (Tahirov et al., 2001). In contrast, the conformation of Ets1, particularly the N-terminal extension from the Ets domain, which was confirmed to be unaffected by crystal packing, exhibits remarkable differences from that of Ets1 structures reported so far. These findings have motivated us to investigate how cooperative DNA binding can be accomplished without any specific interactions between Ets1 and Runx1, and to explore whether the altered conformation of Ets1 has a functional role.

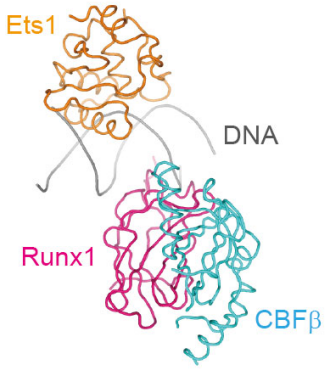

Figure 1. Overall crystal structure of the Ets1–Runx1–CBFβ–DNA quaternary complex. Ets1, Runx1 and CBFβ are colored orange, magenta and cyan, respectively. The DNA molecule is shown as gray tubes.

References

[1] TH. Tahirov et al., Cell, 104, 755 (2001).

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