Crystallographic Analyses of the Runx-1/AML1 (Runt domain) - DNA Interaction and Its Allosteric Control by CBFβ

The promoter regions of most genes contain binding sites for multiple transcription factors whose interactions are essential for the correct regulation of transcription. Such interactions include those between different transcription factors as well as those between subunits of the same factor. In the case of some heterodimeric factors (*e.g.*, Jun/Fos and Myc/Max) both subunits bind to specific DNA sequences, and recent X-ray studies of this type of protein have clarified the molecular mechanisms of their DNA binding (*e.g.*, the "scissors grip" by the Fos-Jun heterodimer). On the other hand, some transcription factors consist of one subunit that binds to DNA and one that does not. In most cases of this type of protein, it remains unknown how a non DNA-binding subunit contributes to DNA binding and whether the mechanism by which a non-DNA-binding subunit stimulates DNA binding is shared with different transcription factors. In this type, very little structural information is available to date, *e.g.* the GABPα-GABPβ-DNA complex *(GABP: GA-binding protein). One well-studied* example of this type of transcription factor is the core binding factor (CBF), which functions as a heterodimer consisting of a DNA-binding α subunit and a non-binding β subunit.

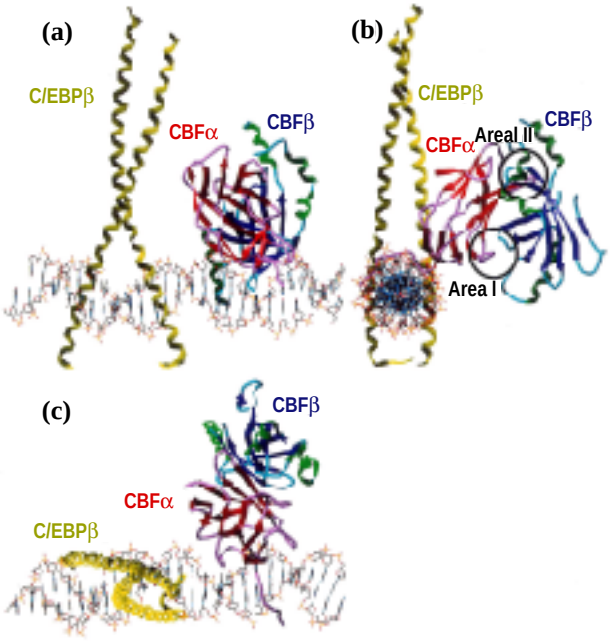

*Fig. 1. Overviews of the CBFα-β-C/EBPβ-DNA quaternary complex from three perspectives [1]. Views (**a**) from the front, (**b**) from the side and (**c**) from the top. Within CBFα, β strands and loops are depicted as red arrows and pink tubes, respectively; within CBFβ, α helices, β strands and loops are depicted as green ribbons, blue arrows and cyan tubes, respectively. The C-terminal region of the C/EBPβ homodimer containing the bZip domain is shown as yellow ribbons. Areas I and II are marked with circles.*

The CBF heterodimeric transcription factors comprised of AML/CBFA/PEBP2α/Runx and CBFβ/PEBP2β subunits are essential for differentiation of hematopoietic and bone cells, and their mutation is intimately related to the development of acute leukemia and cleidocranial dysplasia; (AML: acute myelogenous leukemia, CBFA: core-binding factor A and PEBP: polyomavirus enhancer-binding protein). Here we present the crystal structures of the AML1/Runx-1/CBFα(Runt domain)-CBFβ(core domain)-C/EBPβ(bZip)-DNA, AML1/Runx-1/CBFα(Runt domain)-C/EBPβ(bZip)-DNA, and AML1/Runx-1/CBFα(Runt domain)-DNA complexes, which are abbreviated to CBFα-β-C/EBPβ-DNA, CBFα-C/EBPβ-DNA and CBFα-DNA respectively, obtained from the diffraction experiments at beamlines **BL41XU** and **BL45XU** [1]. Our focus is on the recognition of specific DNA sequences by CBFα and on the mechanism by which CBFβ facilitates DNA binding.

The structure of a complex comprising the CBFα Runt domain and the CBFβ core domain bound to a 26-bp DNA fragment from the CSF-1R promoter, together with the C/EBPβ basic leucine zipper region homodimer, is shown in Fig. 1(a-c). Note that the structures of DNA-bound CBFα are

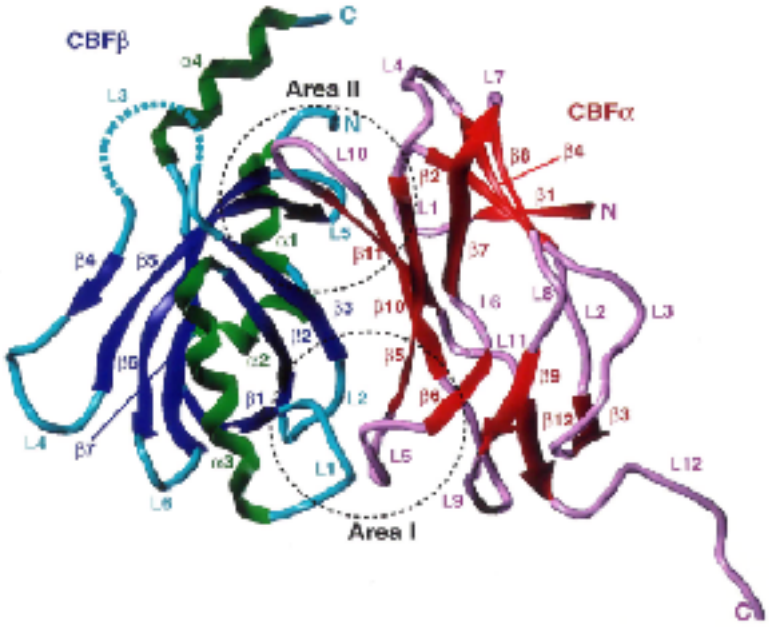

Fig. 2. CBFα-CBFβ heterodimer structure in complex with the DNA [1]. Two predominantly hydrophilic interaction areas between CBFα and CBFβ (Areas I and II) are marked with dotted circles.

very similar, with or without CBFβ: the r.m.s. deviation of the α carbon atoms was only about 0.28 Å. Both the CBFα Runt domain and the CBFβ core domain fold principally as β barrel architectures, with the structure of the former being classified as an s-type immunoglobulin fold. The Runt domain recognizes specific bases (TGTGGTT) in both the major and minor grooves of the DNA, mainly using loops. The CBFβ core domain interacts with the CBFα Runt domain at a site distant from the protein-DNA interface. The CBFα-CBFβ interface extends linearly from the DNA and can be divided into two predominantly hydrophilic interaction areas – one proximal to DNA (Area I) and one distal (Area II) – with an intervening hydrophobic area (Figs. 2 and 1b). Contrary to earlier protein-protein and protein-DNA binding assays [2,3], analyses of crystals of the CBFα-β-C/ EBPβ-DNA and CBFα-C/EBPβ-DNA complexes revealed no direct interaction between the C/EBPβ basic leucine zipper domain and the CBFα Runt domain or the CBFβ core domain.

CBFα-DNA binding is enhanced several folds in the presence of CBFβ. To examine the regulation of CBFα-DNA binding by CBFβ, we compared the structure of the CBFα-β-C/EBPβ-DNA complex with those of the CBFα-C/EBPβ-DNA and CBFα-DNA complexes, which lack the CBFβ subunit. Somewhat surprisingly, CBFβ binding causes no dramatic structural changes in the CBFα Runt domain, which implies that CBFβ does not alter the

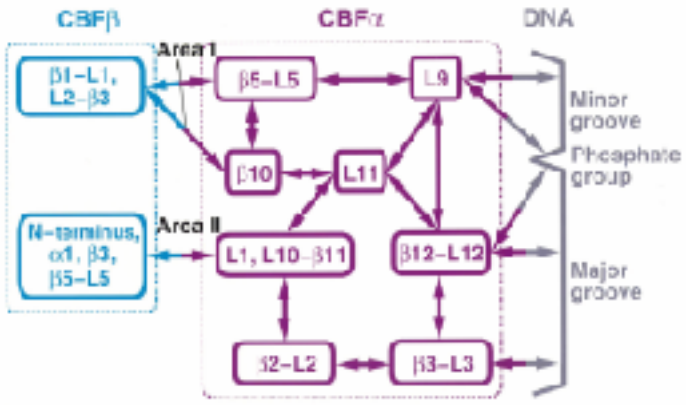

Fig. 3. Schematic representation of the stabilization network between CBFβ (cyan), CBFα (pink) and DNA (gray) [1].

overall conformation of the CBFα Runt domain, but only stabilizes it in a conformation suitable for DNA binding. As described above, CBFβ interacts with CBFα via two predominantly hydrophilic regions– Areas I and II, and their stabilization affects the conformations of the flexible loops in contact with the DNA to grip the DNA molecule at both the major and minor grooves (Fig. 3).

Acute leukemia and related diseases associated with biallelic and heterozygous point mutations of AML1/Runx-1 have been reported. In the case of biallelic mutations, no functional AML1/Runx-1 allele remains; in the case of heterozygotes, the DNA binding activity of AML1/Runx-1 from one allele is impaired, with AML1/Runx-1-CBFβ heterodimerization activity retained. Based on the three-dimensional structure of the CBFα-CBFβ heterodimer bound to the DNA, point mutation sites in the heterozygous cases are mapped exclusively to regions of the CBFα-DNA interface (Fig. 4). It is readily apparent that these mutations impair the capacity of CBFα to bind to DNA without affecting the overall folding architecture of CBFα or CBFα-CBFβ heterodimerization, which could explain their dominant negative behavior.

Acute Myelogenous Leukemia and related diseases

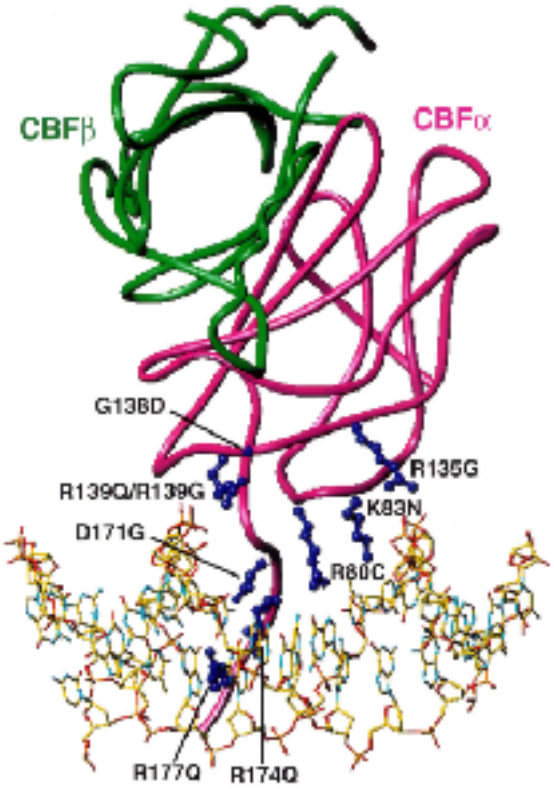

Fig. 4. Structural maps of CBFα point mutations occurring in acute myelogenous leukemia and related diseases [1]. The mutated residues are shown in green ball and stick presentations. The peptide backbones of CBFα and CBFβ are depicted as pink and cyan tubes, respectively.

Tahir H. Tahirov[a,b], Taiko Inoue-Bungo[a] and Kazuhiro Ogata[a,b,c]

(a) Kanagawa Academy of Science and Technology (KAST)
(b) SPring-8 / RIKEN
(c) Yokohama City University School of Medicine

E-mail: ogata@med.yokohama-cu.ac.jp

References

[1] T.H. Tahirov, T. Inoue, M. Sasaki, K. Kimura, H. Morii, A. Fujikawa, M. Shiina, K. Sato, T. Kumasaka, M. Yamamoto, S. Ishii, K. Ogata, Cell **104** (2001) 755.

[2] D.E. Zhang *et al.*, Mol. Cell. Biol. **16** (1996) 1231.

[3] M.S. Petrovick *et al.*, Mol. Cell. Biol. **18** (1998) 3915.