Assembly of Transcriptional Regulatory Factors, c-Myb and C/EBPβ from Separated Sites on a Promoter

Assembly of stereospecific, multiprotein complexes on enhancers and promoters is a key step in transcriptional activation. Recent X-ray analyses of high-order complexes comprised of transcription factors bound to DNA have concentrated exclusively on cases in which interactions between transcription factors enhance their cooperative binding to adjacent sites on a promoter. In many eukaryotic genes, however, transcription factors bind to promoters at sites distant from one another, yet act synergistically to activate transcription. It has been proposed that DNA looping mediated by their interaction brings transcription factors scattered along the DNA into sufficiently close proximity to enable them to form nucleoprotein complexes. Here we address this problem in the case of the synergistic *trans*-activation of myeloid genes by c-Myb and C/EBPβ bound distantly from each other to the DNA .

The c-Myb transcriptional regulator is involved in the proliferation and differentiation of hematopoietic cells. Specifically, it cooperates with a C/EBP (CAAT/enhancer-binding protein) family member to induce myeloid cell differentiation. c-Myb has three tandem subdomains (R1, R2 and R3), each bearing a helix-turn-helix (HTH)-related motif, in its DNA-binding domain [1,2], whereas C/EBP family members contain a bZip-type motif for DNA binding. The *mim-1* promoter is a well-characterized c-Myb target gene promoter and this gene is a marker for granulocyte differentiation. Its promoter region includes the binding sites for c-Myb and C/EBP family members, which are critical for transcriptional regulation, separated by an intervening sequence of ~ 80 base pairs. Avian myeloblastosis virus (AMV) v-Myb, an oncogenic mutant of c-Myb, has no synergistic capacity with C/EBP for *trans*-activation of the *mim-1* gene, resulting in the inhibition of granulocyte differentiation and induction of leukemia.

To establish the structural basis of the *trans*-activational synergy between c-Myb and C/EBP family members, we analyzed the complex structures including c-Myb or AMV v-Myb, C/EBPβ and the promoter DNA. Because the X-ray crystallographic analysis of a DNA-loop-containing multiprotein –DNA complex seemed to be quite difficult, combinational X-ray crystallography experiments and atomic force microscopy (AFM) analyses were carried out [3]. X-ray diffraction data were collected on a RIKEN beamline **BL45XU** [4]. In the c-Myb (R1R2R3)–C/EBPβ (bZip)–DNA complex (Fig. 1), c-Myb–C/EBPβ intercomplex interactions were observed between the R2 subdomain of c-Myb bound to one DNA fragment and the C-terminal leucine-zipper part of C/EBPβ bound to another DNA fragment. On the c-Myb side, a hydrogen bond between a backbone amide of c-Myb R2 and a DNA minor groove phosphate, which plays an important role in the c-Myb–DNA interaction [5,6], becomes stabilized by a hydrogen-bonding network provided by C/EBPβ binding. These findings led us to assume an interaction between c-Myb and C/EBPβ separately bound to the native promoter DNA, with an intervening DNA loop, as shown in Fig. 2, where the *mim-1* promoter was used as a native promoter.

The oncogenic mutation points noted in the AMV v-Myb map near the interaction site with C/EBPβ, and the c-Myb–C/EBPβ interactions were disrupted in the crystal of the AMV v-Myb (truncated R1R2R3)–C/EBPβ(bZip)–DNA complex [3]. The data on Myb–C/EBPβ

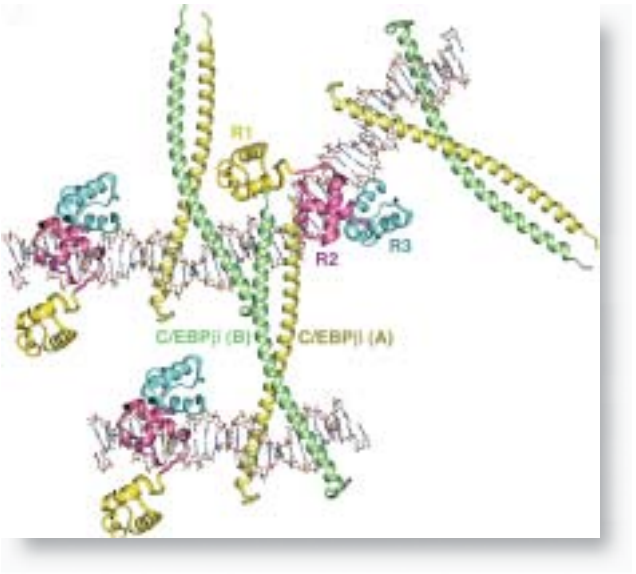

Fig. 1. Overview of three closely packed c-Myb–C/EBPβ–DNA complex molecules [3]. α-helical regions within the proteins are shown as ribbons; the remaining parts are shown as tubes. c-Myb R1, R2 and R3 and C/EBPβ chains A and B are respectively in dark yellow, pink, blue, yellow and green. The DNA molecules are shown in a stick representation.

interactions in crystals were consistent with those from the GST pull-down experiments in solution using GST-fused c-Myb or AMV v-Myb, and radioisotope-labeled C/EBPβ or its mutants [3]. The proposed DNA loop formation in the c-Myb–C/EBPβ–*mim-1* DNA complex was confirmed by using the AFM method. The AFM observations indicated that about 75% of the c-Myb and C/EBPβ-bound *mim-1* promoter DNA exhibited DNA looping, whereas the AMV v-Myb and C/EBPβ-bound *mim-1* promoter DNA had no DNA looping (Fig. 3). From the luciferase *trans*-activation assays, the aforementioned c-Myb–C/EBPβ interaction was shown to be critical for the cooperative *trans*-activation of the *mim-1* gene by c-Myb and C/EBPβ [3].

In transcriptional regulation, two factors are considered to be involved: the stabilization of the regulatory factor–DNA complex and the stereospecific assembly of these proteins on the looped or deformed DNA. For the stabilization of protein–DNA interactions, a direction-sensitive protein backbone amide–DNA phosphate hydrogen bond, whose formation depends on the protein conformation and which is surrounded by the sidechains of adjacent residues interacting with DNA minor groove atoms and/or partner protein atoms, seems to be critical [5,6]. For the stereospecific assembly of regulatory factors, particularly protein-induced DNA looping, there are few examples for which structure/function relationships have been analyzed. The present study provides important clues that increase our understanding of the regulation of *trans*-activation mediated by distantly bound transcription factors and the dysregulation caused by oncogenic mutations. It is expected that extensive studies in the future will elucidate the entire molecular mechanism of enhanceosome or repressosome formation.

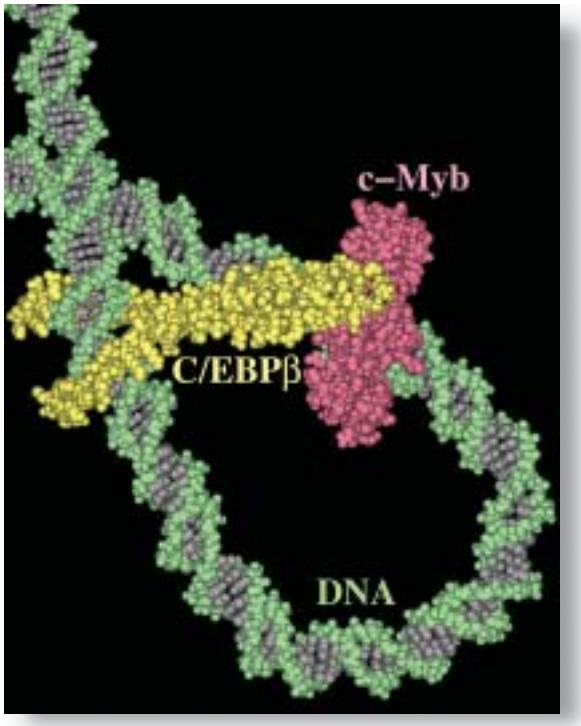

Fig. 2. A modeled structure of the complex composed of c-Myb, C/EBPβ and the *mim-1* promoter DNA, showing DNA loop formation [3,6].

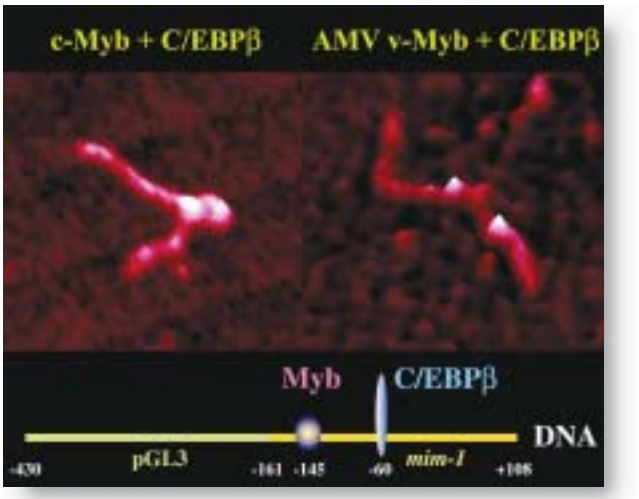

Fig. 3. Representative AFM images of *mim-1* promoter DNA complexes with c-Myb and C/EBPβ (left), and with AMV v-Myb and C/EBPβ (right) [3,6]. The bottom scheme shows the DNA construct used for the AFM experiments. The *mim-1* promoter with the separated c-Myb and C/EBPβ-binding sites was fused to the pGL3 vector.

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References

[1] K. Ogata *et al.*: Cell **79** (1994) 639.
[2] K. Ogata *et al.*: Nature Struct. Biol. **2** (1995) 309.
[3] T.H. Tahirov *et al.*: Cell **108** (2002) 57.
[4] T.H. Tahirov *et al.*: Acta Cryst. D **57** (2001) 1655.
[5] T.H. Tahirov *et al.*: Cell **104** (2001) 755.
[6] K. Ogata, K. Sato and T.H. Tahirov: Curr. Opin. Struct. Biol. **13** (2003) 40.