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Structural analysis of Spi-B DNA-binding Ets domain in complex with target DNA

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1 Introduction

Plasmacytoid dendritic cells (pDCs) produce large amounts of type-I interferon (IFN-I) upon sensing nucleic acid components of pathogens by Toll-like receptors (TLR7 and TLR9). The transcription factor Spi-B has the DNA-binding Ets domain, and transactivates the *Ifna4* promoter co-operatively with another transcription factor, IFN regulatory factor-7 (IRF-7), for IFN-I production in pDCs. Spi-B associates with IRF-7, and activates transcription by binding to the 5'-AGAA-3' sequence, being different from 5'-GGAA-3', known as the Ets domain recognition sequence. To elucidate the molecular mechanism for the co-operative transactivation of the *Ifna4* promoter by Spi-B and IRF-7, we carried out X-ray structural determination of the Spi-B Ets domain in complex with target DNAs, including 5'-AGAA-3' and 5'-GGAA-3' sequences.

2 Experiment

Protein and DNA solutions were mixed to a 1:1 molar ratio and incubated on ice for more than three hours. Crystals of the complex were grown at 20°C in a droplet mixed with 0.75 µL of the complex solution and 0.75 µL of the reservoir solution (0.1 M MES pH 5.6, 7.6% (w/v) PEG8000, 8.0% (v/v) ethylene glycol) using the sitting drop-vapor diffusion method. X-ray diffraction data collection were performed at 100 K using a PILATUS3 S6M detector system on PF-BL5A beam line in KEK (Tsukuba, Japan). Diffraction data were processed using the programs XDS and CCP4. The structures were determined by the molecular replacement method with the program MOLREP, using an X-ray structure of the PU.1 Ets domain (PDB ID: 1PUE).

3 Results and Discussion

Overall structure of the Spi-B Ets domain in complex with the target DNA is shown in Fig. 1A. Spi-B Ets domain has four helices (H1 – H4) and a β-sheet composed of four anti-parallel β-strands (B1 – B4), and the long helix H3 enters the major groove of DNA to interact with the base pairs of the recognition sequence. The position of the first A-T base pair of the recognition sequence is numbered as (0), and the base pairs on the 3' and 5' sides are as (1) and (-1), respectively. The binding of the Spi-B Ets domain induces a kink in DNA at (0) – (1), as shown in Fig. 1A. The angles between the helical axes of DNA (-7) – (0) and (1) – (7) are 42.1° (AGAA) and 37.3° (GGAA), indicating that AGAA is more kinked by 4.8° than GGAA.

Since it was reported that Spi-B associates with IRF-7 through its Ets domain to transactivate the *Ifna4* promoter, the relative position of the recognition sequences of these two proteins should be important. The IRF-7 recognition site is 12 base pairs upstream from the Spi-B recognition site, so the two proteins bind on the same side of the DNA (Fig.1B). The Spi-B-induced kinked DNA structure at 5'-AGAA-3' (position (0) – (-1)) would be favorable for the Spi-B Ets domain and IRF-7 to approach each other. Thus, it could be proposed that the Spi-B Ets domain recognizes the 5'-AGAA-3' sequence to induce a large kink in DNA for the association with IRF-7 (Fig. 1B). This may be a reason why Spi-B recognizes and binds to the 5'-AGAA-3' sequence in the *Ifna*4 promoter rather than the well-known sequence of 5'-GGAA-3', and may be essential for the co-operative transactivation of the *Ifna4* promoter by Spi-B and IRF-7.

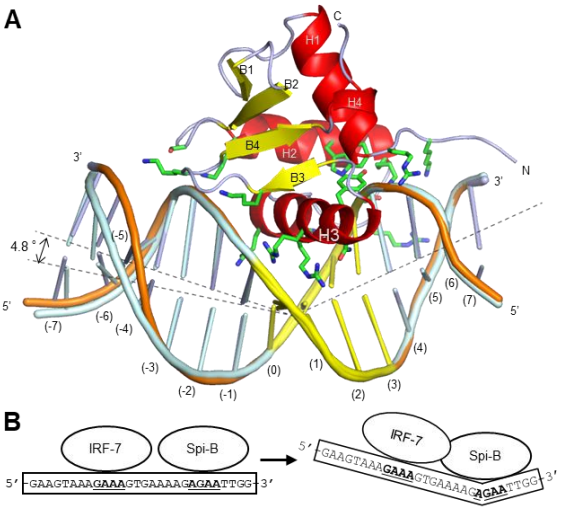

Fig. 1: (A) Overall structure of the Spi-B/DNA complex is illustrated. Amino acid residues of Spi-B interacting with DNA are shown by a stick model. DNAs of AGAA (orange) and GGAA (cyan) are superimposed, and the recognition sequences are shown in yellow. Helical axes of DNA ((0) – (-7) and (1) – (7)) are indicated by dotted lines. (B) Schematic drawing shows that the Spi-B-induced kinked DNA structure is favorable for Spi-B and IRF-7 to approach each other for association on the *Ifna4* promoter.

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References

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