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Structural analysis of the Nrf2-containing enhanceosome that regulates oxidative stress responsive genes

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1 Introduction

Living cells are constantly exposed to oxidative stress, which damages biological molecules, leading to aging and diseases including cancer. Under an oxidative stress condition, anti-oxidant gene expression is induced by the transcription factor Nrf2 to maintain homeostasis of the cellular redox state. Responding to oxidative stress, Nrf2 moves into the nucleus and binds target genes as a heterodimer with a small Maf (sMaf) protein to transactivate the target genes.

Nrf2 belongs to the basic leucine-zipper protein having an accessory domain called the cap 'n' collar (CNC) domain at the N-terminal side of the basic region (CNC-bZIP). Nrf2 heterodimerizes with sMaf and specifically binds an antioxidant responsive element (ARE) in the target gene enhancers. ARE contains 10 base pairs of DNA, which are longer than canonical bZIP binding sequences. The recognition mechanisms for ARE by the Nrf2-sMaf heterodimer remains unclear at the molecular structural level because of the lack of structural information on the Nrf2-sMaf-DNA complex. Here we present the crystal structure of the Nrf2-MafG-DNA complex formed on the *NQO1* enhancer. This is the first high-resolution structure of a heterodimeric proteins-DNA complex containing the CNC-type bZIP protein.

2 Experiment

The bacterially expressed human Nrf2 fragment corresponding to CNC-bZIP was purified by ion-exchange, hydrophobic, and size exclusion chromatographies. The fractions were checked by SDS-PAGE. The collected sample was buffer-exchanged to distilled water containing 5 mM DTT by ultrafiltration. The MafG fragment was prepared as described previously [1]. Both strands of the 15 bp double-stranded DNA (dsDNA) fragments containing the ARE sequence were purchased from FASMAC and annealed. The dsDNA fragment was purified by hydroxyapatite chromatography.

The Nrf2-MafG-DNA complex was prepared by mixing the equimolar amount of Nrf2, MafG, and dsDNA fragments.

Initial crystallization screening was performed by the sitting drop method with Natrix 1 and 2 (Hampton Research). The several hit conditions were further optimized with a 24-well-formatted plate. The obtained crystals were dipped in a cryo solution supplemented with 30% glycerol and flush-cooled in a liquid nitrogen stream.

High-resolution diffraction images were obtained at BL-5A in Tsukuba PF. The obtained images were indexed, integrated, and scaled with HKL2000. The phase was determined by molecular replacement with the homodimeric MafG-DNA complex [1].

3 Results and Discussion

The solved crystal structure of the heterodimeric CNC-bZIP protein-DNA complex revealed the mechanism of ARE recognition by Nrf2 and sMaf (Fig. 1) (manuscript *in preparation*). In the Nrf2 part, the CNC domain does not interact with DNA directly but affects the orientation of the basic region of Nrf2 against the DNA, enabling the specific recognition of ARE. Recently, aberrant activation of Nrf2 in cancer cells reportedly promotes survival and progression of the cells, and thus an anti-Nrf2 drug is considered as a promising anti-cancer drug. The solved structure would contribute to a rational design for the anti-Nrf2 drug especially targeting DNA-binding activity of Nrf2.

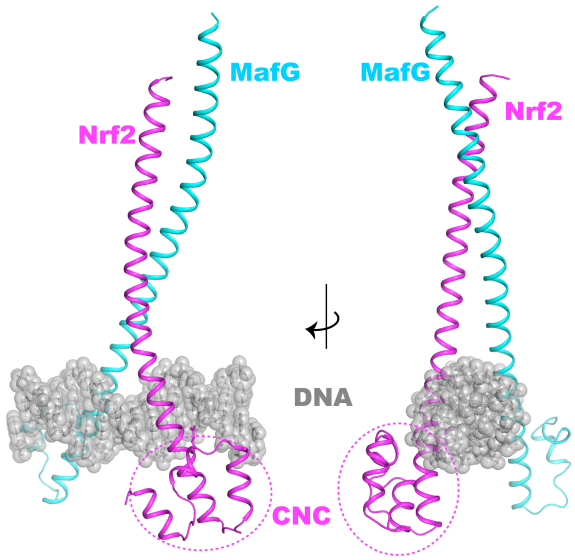

Fig. 1: The crystal structure of the Nrf2-sMaf-DNA complex on the *NQO1* enhancer.

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References

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