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Structural and functional analysis of a highly conserved hypothetical protein, TTHA1606

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Nif3 family proteins are widespread across species and have a high degree of conservation from bacteria to mammals. This highly conservation suggests that proteins in this family play an important role in life process. However, the cellular and molecular function remains to be elucidated. In this study, we determined the structure of TTHA1606 by the X-ray crystallography and the transmission electron microscopy analysis, and we studied the DNA-binding ability by electrophoretic mobility shift assay (EMSA). The determined crystal structure showed that TTHA1606 forms hexamer and the overall shape of the hexamer is viewed as a double-ring of two trimers (Figure 1). This ring structure was also observed by transmission electron microscopy. The inside diameter of the ring is approximately 24Å, which is large enough to allow single-stranded DNA (ssDNA) to pass through the ring, but not for double-stranded DNA (dsDNA). The result of EMSA actually showed that TTHA1606 bound to ssDNA but not dsDNA. This is the first report of a Nif3 family protein that can bind to DNA. In addition, the crystal structure showed that there are two cavities inside the ring (Figure 2). One of the cavities has been reported to be a metal-ion binding site, and another cavity is thought to be an unknown-ligand binding site. These results suggest that the inside of the ring is important in the function of TTHA1606, that ma be related to DNA transaction.

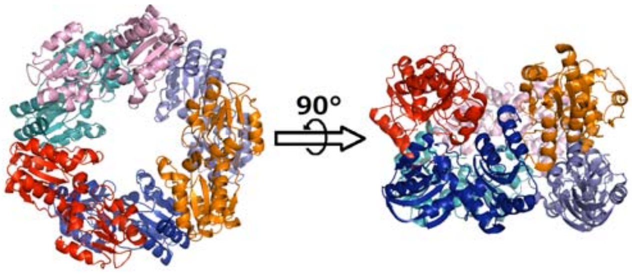

Figure 1 The haxameric structure of TTHA1606

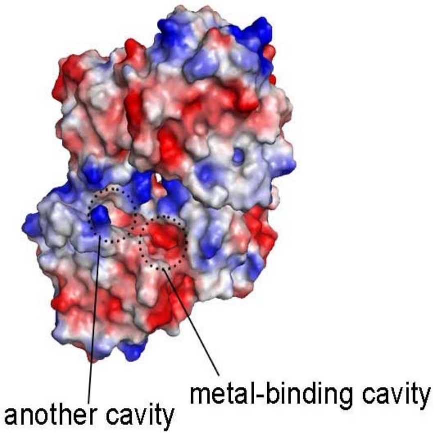

Figure 2 Two cavities inside the ring (viewed as a dimmer)

Reference

[1] Tomoike, F., Wakamatsu, T., Nakagawa, N., Kuramitsu, S. and Masui, R. (2009) *Proteins* **76**, 244-248