

Structure and function of the transcriptional repressor CsoR from *Thermus thermophilus* HB8

Keiko Sakamoto¹, Yoshihiro Agari¹, Kazuko Agari¹, Seiki Kuramitsu^{1, 2}, Akeo Shinkai¹

(¹RIKEN SPring-8 Center, Harima Inst. ²Grad. Sch. of Sci., Osaka Univ.)

e-mail: k-saka@spring8.or.jp

Metal ions are essential for living organisms but they are very toxic in excess concentrations; therefore many organisms have to keep appropriate cellular metal-ion concentration. Metal-ion transporters, which efflux or influx metal-ions, control the metal-ion concentration in response to the environmental alterations. In many cases, the expression of bacterial transporters is transcriptionally regulated.

TTHA1719 from *T. thermophilus* HB8 was identified as a homolog of the copper sensing transcriptional repressor (CsoR), a transcriptional regulator which regulates the expression of the copper-ion transporter gene. *In vitro* transcription assays showed that, in the absence of copper ions, TTHA1719 bound to the promoter region of the copper-sensitive operon *copZ-csoR-copA* containing copper transporter-like gene, and repressed the expression of the operon, while, in the presence of copper ions, TTHA1719 lost the ability to repress transcription (Fig. 1). We investigated the alternation of the mRNA expression of the *T. thermophilus* HB8 by DNA microarray analysis. The expression of the *copZ-csoR-copA* operon increased after cultivation in the presence of CuSO₄. On the other hand, in the Δ TTHA1719 stain, a deletion mutant of the TTHA1719 gene, the expression of *copZ* increased even though in the absence of CuSO₄ as compared with that in the wild-type stain. These results indicate that TTHA1719 is a CsoR. *T. thermophilus* CsoR could bind both Cu(I) and Cu(II). Furthermore, other metal ions such as Zn(II), Ag(I), Cd(II) and Ni(II) had effects on transcriptional derepression of *T. thermophilus* CsoR. The copper ion-binding motifs of most CsoR-family proteins contain C-H-C, whereas the corresponding residues of *T.*

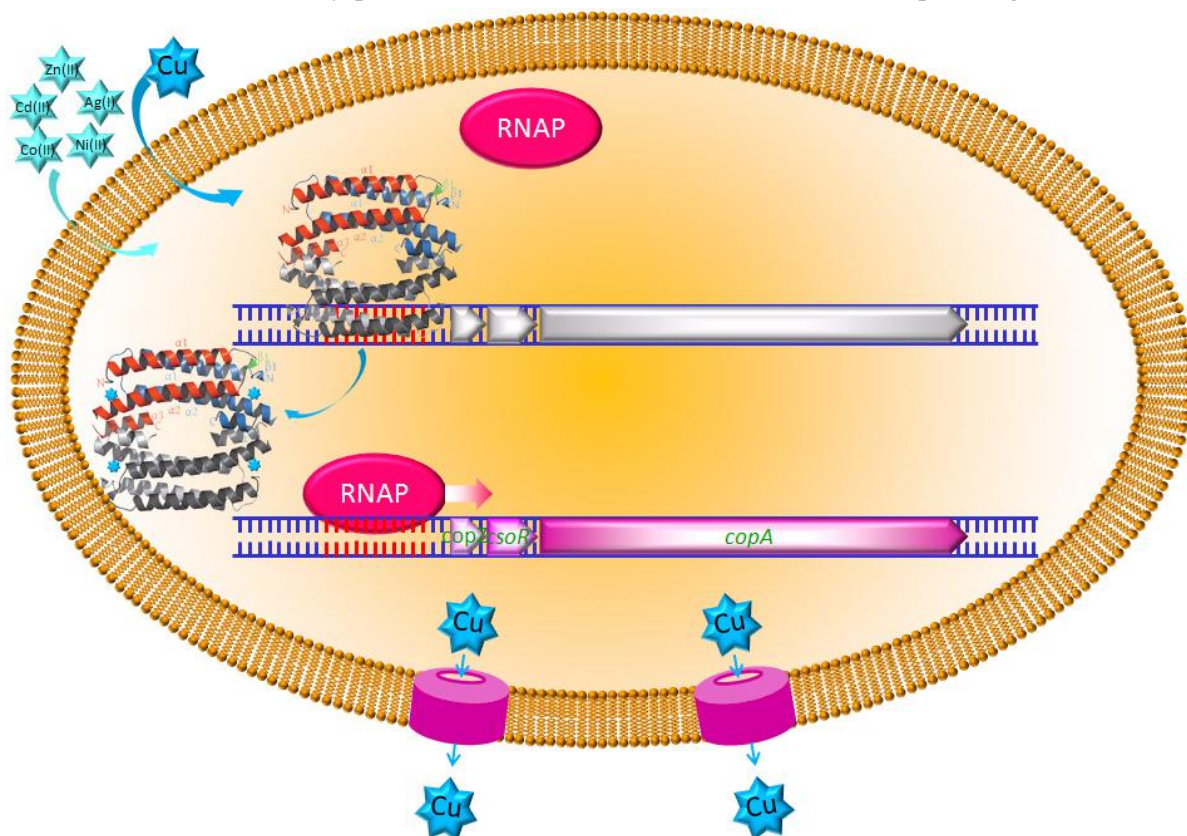


Fig. 1. Activity of *T. thermophilus* CsoR

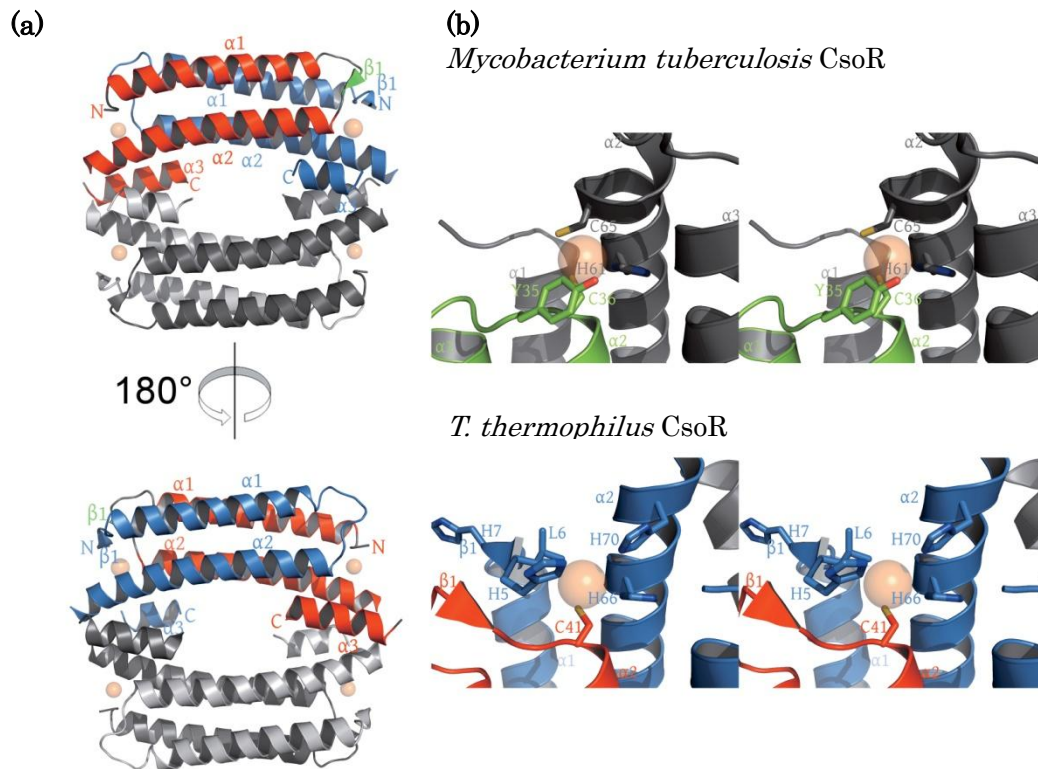


Fig. 2. X-ray crystal structure of *T. thermophilus* CsoR. (a) Overall structure of *T. thermophilus* CsoR tetramer. (b) Copper binding sites of *Mycobacterium tuberculosis* CsoR and those of *T. thermophilus* CsoR. Copper ions are indicated by orange spheres.

thermophilus CsoR are C-H-H. X-ray crystal structure analysis revealed that *T. thermophilus* CsoR forms a homotetramer (Fig. 2a). Although the overall structure is similar to that of *Mycobacterium tuberculosis* CsoR, an N-terminal histidine residue of *T. thermophilus* CsoR was likely to be involved in metal binding unlike in the case of *Mycobacterium tuberculosis* CsoR, i.e., the metal-binding motif of *T. thermophilus* CsoR is possibly H-C-H-H (Fig. 2b). If so, the motif is the same as that of *E. coli* RcnR, which binds to Ni(II)/Co(II) and transcriptionally regulates the expression of the cognate transporter. Interestingly, when unconserved His70 in the metal-binding motif of *T. thermophilus* CsoR mutated to cysteine, its metal-binding specificity changed and its DNA-binding affinity increased. These results indicate that the His70 of *T. thermophilus* CsoR is important to determine the specificity for metal ions and DNA.

Reference

Sakamoto *et al.* (2010) *Microbiology* **156**, 1993-2005.