Structure and Function of Stationary-Phase-Dependent Regulatory Protein from *Thermus thermophilus* HB8

In order to survive, organisms have to respond to changes in environmental conditions. These switches are mediated by alternative σ factors of RNA polymerase or transcription factors, which regulate the transcription of genes to respond to changes. Bacterial cells enter the stationary phase upon nutrient depletion. During the stationary phase, the gene expression pattern changes globally, and genes that are required for adaptation and survival, including those involved in nutrient scavenging, DNA repair, protein turnover, and protection from oxidative damage, are expressed. We performed structural and functional analyses of the transcriptional regulator SdrP (stationary-phase-dependent regulatory protein) from an extremely thermophilic bacterium *Thermus thermophilus* HB8, the mRNA expression of which increases in the stationary phase [1] (Fig. 1). Using an *sdrP*-deficient strain, we found that the strain showed growth defects, particularly when grown in a synthetic medium, and increased sensitivity to disulfide stresses although the gene was nonessential. The expression of several genes was altered in the *sdrP* disruptant. Among them, we found eight SdrP-dependent promoters using *in vitro* transcription assays. The transcription activation *in vitro* was independent of any added effector molecule. On the basis of amino acid sequences and three-dimensional structures of the protein products of the genes regulated by these promoters, we speculate that they are involved in activities such as securing nutrient and energy supply, and protecting against oxidative damage to DNA [1] (Fig. 1).

The *T. thermophilus* SdrP belongs to cyclic AMP (cAMP) receptor protein (CRP), also referred to as catabolite activator protein, CAP/fumarate and nitrate reduction regulator (FNR) superfamily proteins, which are global transcriptional regulators widely distributed in bacteria and predominantly function as activators. In many cases, CRP/FNR regulators respond to a wide range of endogenous and exogenous signals such as cAMP, anoxia, redox state, oxidative and nitrosative stress, nitric oxide, carbon monoxide, 2-oxoglutarate, and temperature [2]. The cAMP-dependent regulatory mechanism of CRP has been extensively studied for *E. coli* CRP, a prototype of this family of proteins, in which two cAMP binding sites are present in each monomer [2,3]. *E. coli* CRP is a homodimer that contains a helix-turn-helix DNA-binding motif in its C-terminal domain. CRP undergoes conformational change upon cAMP binding

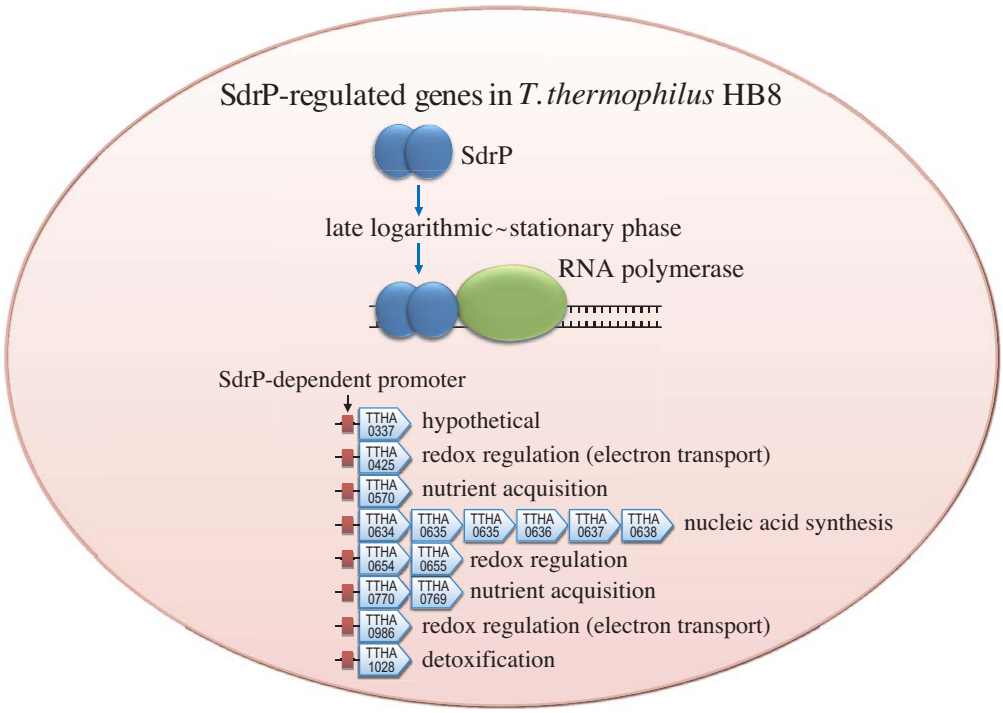

Fig. 1. Schematic representation of the activity of the *T. thermophilus* SdrP. The SdrP-regulated genes and their functions are indicated.

to the primary binding site in its N-terminal domain, and the CRP-cAMP complex interacts with a 22-bp DNA site exhibiting twofold symmetry that has the consensus sequence 5'-AAATGTGATCTAGATCACATTT-3' [4]. The N-terminal domain of *T. thermophilus* SdrP consists of one α-helix and eight β-strands that adopt a double-stranded β-helix fold with a jelly roll topology (Fig. 2(a)). The C-terminal domain of *T. thermophilus* SdrP consists of four α-helices and four β-strands that adopt a winged helix-turn-helix fold (Fig. 2(a)). These two domains are connected by a large α-helix as the linker. The three-dimensional structure of *T. thermophilus* SdrP is similar to that of the DNA-binding form of *E. coli* CRP [3]—the form complexed with cAMP and DNA—and has an r.m.s.d. of 2.3 Å (Fig. 2(b)). Superpositioning of the structure of SdrP with that of *E. coli* CRP revealed that residues G72, E73, L74, R83, S84, T128, and S129 of *E. coli* CRP, which are primary cAMP-binding sites, correspond to G59, E60, E61, R68, Y70, A106, and Y107 in SdrP (Fig. 2(c,d)). It should be noted that the side chains of E60, E61, Y70, and Y107 of SdrP penetrate into a space corresponding to the cAMP-binding pocket of *E. coli* CRP (Fig. 2(c)). The structure of SdrP suggests that cAMP cannot enter the site corresponding to the primary cAMP-binding site of *E. coli* CRP owing to steric hindrance by bulky residues. These structural properties of SdrP imply that this protein does not require an effector molecule to bind DNA, which is supported by the observation that this protein can positively regulate transcription independent of any effector molecule *in vitro*. Y70 of SdrP, which probably causes steric hindrance in cAMP binding, corresponds to S84 of *E. coli* CRP and possibly S86 of *T. thermophilus* CRP. At this position, a small residue might be necessary for a CRP family protein to act as a cAMP-dependent transcriptional regulator.

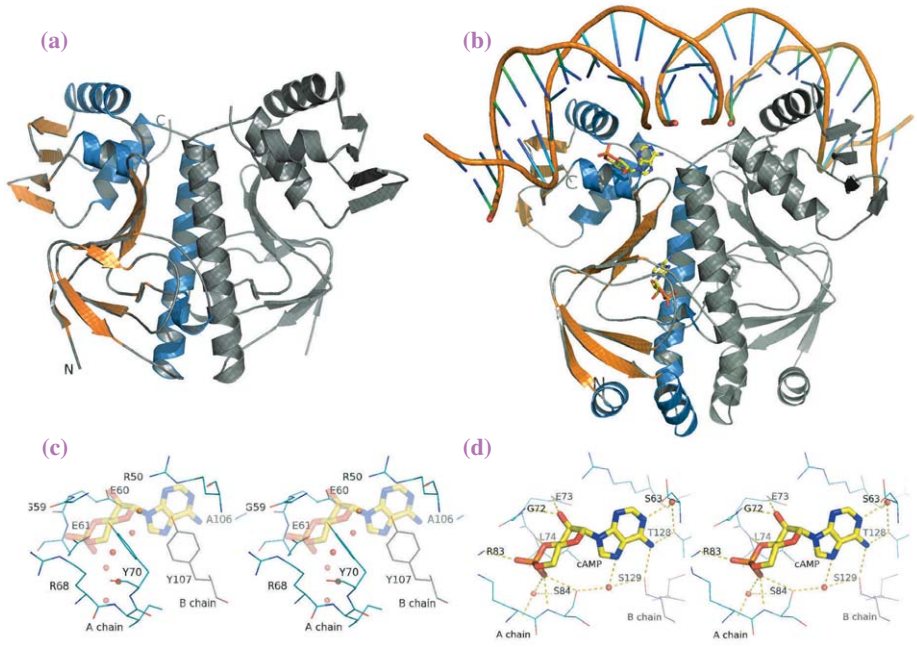

Fig. 2. **(a)** Ribbon diagram of the *T. thermophilus* SdrP dimer. The α-helices and β-strands in one chain are colored blue and orange, respectively, and the other chain is colored dark gray. **(b)** Ribbon diagram of the *E. coli* CRP-cAMP-DNA complex [3]. The color scheme is the same as in **(a)**. cAMP molecules are shown as stick models. **(c)** Stereoview of the site in *T. thermophilus* SdrP corresponding to the primary cAMP-binding site of *E. coli* CRP shown in **(d)**. A cAMP molecule of the *E. coli* CRP-cAMP-DNA complex is superimposed on SdrP as a transparent stick model. **(d)** Stereoview of the primary cAMP-binding site in the N-terminal domain of *E. coli* CRP. Residues involved in cAMP binding are labeled. A cAMP molecule is shown as a stick model.

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