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# Study of transcriptional regulatory system of *T. thermophilus* HB8: Regulatory mechanism of CRISPR/*cas* system

Akeo Shinkai
(RIKEN SPring-8 Center, Harima Inst.)
e-mail: ashinkai@spring8.or.jp

Transcription is the first step of the central dogma, and a fundamental process of life. In bacteria, RNA polymerase holo-enzyme containing sigma factor can recognize promoters and start transcription. Transcription initiation is controlled by various kinds of sigma factors and transcription factors that activate or repress transcription of certain gene(s) to adapt to environmental alterations. Elucidation of the function of those regulators is necessary to understand the fundamental cell system (Fig. 1). *T. thermophilus* HB8 is an appropriate model organism to study transcriptional regulatory mechanism because the genome size and number of genes are about half of those of *E. coli* and *Bacillus subtilis* which have been widely used as model organisms, and the number of transcriptional regulator is predicted to be less than those of *E.* coli and *Bacillus subtilis*. Thus, transcriptional regulation of *T. thermophilus* is expected to be essential. However, to my knowledge, most transcription factors from this strain (~80%) have been functionally unidentified. Therefore, we are now trying to identify the function of remaining all transcription factors [1-4, 7,8] because it is necessary to understand the essential and fundamental cell system. On the other hand, clarification of the transcriptional regulatory mechanism will lead to classify ~500 functionally unknown genes remained in this strain, and to obtain clues to elucidate function of them because transcription of several genes sharing similar cellular functions is often synchronously regulated (Fig. 2).

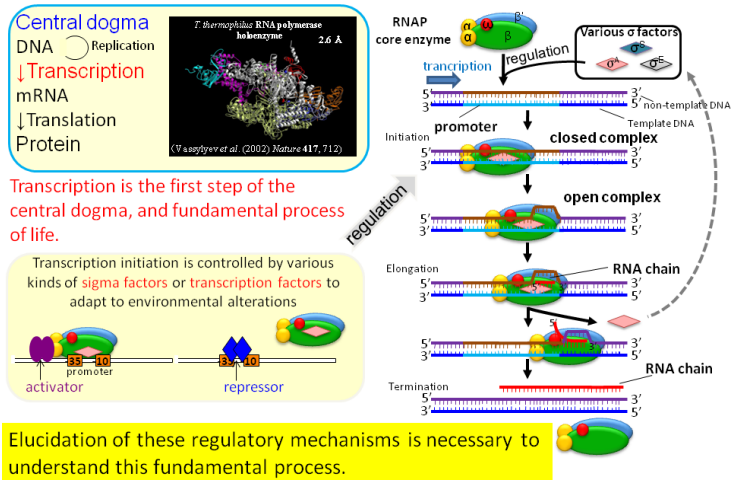


**Fig. 1. Bacterial transcription.**

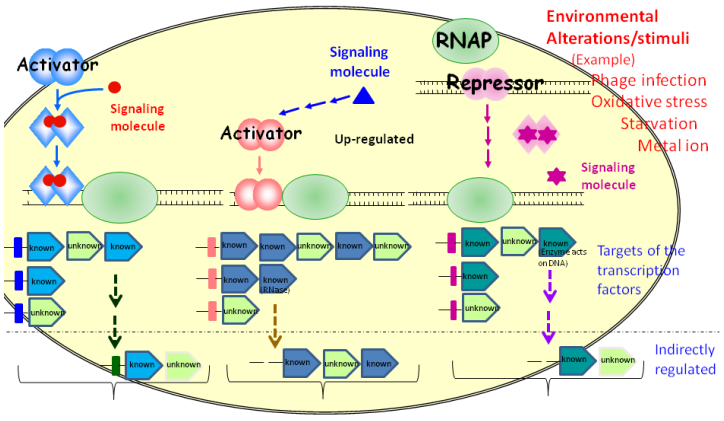


**Fig. 2. Classification of the functionally unknown gene based on the transcriptional regulation.**

We are now focusing on global transcriptional regulators that regulate a lot of genes. We found that one of them (cAMP receptor protein (CRP)) sensed phage infection, and activated transcription of several genes including clustered regularly interspaced short palindromic repeat (CRISPR)-associated (*cas*) ones that comprise a host defense system against foreign replicons (Fig. 3) [1,7]. In this presentation, I will show regulatory mechanism of the CRISPR/*cas* system, as an example in a series of our study. In general, the CRISPR/*cas* systems are composed of CRISPR and *cas* genes. CRISPRs are composed of 24–47 bp direct repeats separated by non-repetitive unique spacer sequences of similar length. Sequences derived from foreign replicons such as phages and plasmids are found in the spacers of several CRISPRs. CRISPR loci are transcribed and processed into small CRISPR RNAs that specify acquired immunity against foreign replicons through a mechanism that relies on the strict identity between CRISPR spacers and targets. If cells do not have any CRISPR spacers that are identical to the sequences of an invading replicon, a fragment derived from the replicon can be incorporated into a CRISPR locus of the cells as a new spacer after infection by the replicon, this phase being designated as the adaptation phase. The new spacer sequence plays a role in immunity against subsequent infection by the same foreign replicon, this phase being designated as the interference phase. Bioinformatical and experimental studies support that transcribed spacer RNAs directly target DNA or RNA of foreign replicons. *T. thermophilus* HB8 has 12 CRISPR loci, and several other *cas* family genes (Fig. 3). In order to investigate the altered expression profiles of the CRISPR/*cas* systems, genome-wide transcription profiling of the strain infected with lytic phage φYS40 was performed by DNA microarray analysis. Interestingly, the expression of most CRP-regulated genes, including two *cas* operons, was most markedly up-regulated, especially around the beginning of host cell lysis, although up-regulation of the *crp* gene was not observed. The up-regulation level for the CRP-regulated genes was decreased in the *crp*-deficient strain. Thus, it was suggested that cAMP is a signaling molecule that transmits the information on phage infection to the CRP to up-regulate those genes. CRP-independent up-regulation of several *cas* genes and that of CRISPRs were observed, suggesting the involvement of unidentified regulatory factor(s) induced by phage infection. On analysis of the expression profile of the entire genome, we could speculate that upon phage infection, the signal was transmitted to the cells, host response systems including CRISPR defense ones being activated, while the overall efficiencies of transcription, translation, and metabolism in the cells decreased.

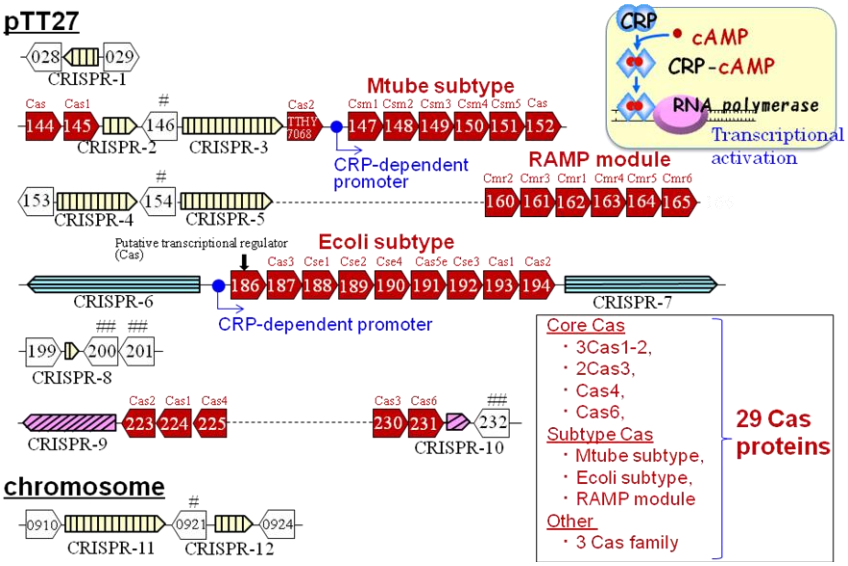


**Fig. 3. CRISPR/*cas* system of *T. thermophilus* HB8.**

We have also performed X-ray crystal structure analysis of *T. thermophilus* Cas proteins [5,6]. These proteins adopt novel folds with large continuous basic patches on one side of its surface, which possibly binds DNA or RNA.

References


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