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INDUCTION MECHANISM OF COLD-STRESS RESPONSIVE RNA HELICASES IN *Thermococcus kodakarensis*

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[Background] Hyperthermophilic archaeon *Thermococcus kodakarensis* possesses cold-stress inducible RNA helicase. It has been reported that *Tk-deaD* in *T. kodakarensis* is involved in an adaptation to the cold stress environments. The regulation mechanism for cold sensing was unclear although the regulatory element which was composed of five-adenosine (AAAAA) repeat was identified in the region between Shine Dalgarno (SD) sequence and initiation codon ATG. Therefore, this study aims to identify factors for cold sensing such as nucleotide sequence, nucleotide length and the location of adenosine cluster. In addition, dependency of binding stability between template DNA and mRNA was also investigated.

[Experimental Method] Expression-probe plasmid pTKR was constructed using thermostable catalase from the *Pyrobaculum calidifontis* as a reporter. The plasmid pTKRD, which contained the BRE, TATA region, and Shine-Dalgarno (SD) region, including the initiation codon of the *Tk-deaD* gene, exhibited cold-inducibility. In the present study, various mutations were introduced into the regulatory region of *Tk-deaD*. Chimeric construct pTKRG with the glutamate dehydrogenase (*gdh*) promoter, whose expression is constitutive independent of culture temperatures was then used for further mutant design. Mutants carrying various length of adenosine cluster between SD and ATG were constructed by site-directed mutagenesis for pTKRG with the corresponding primer sets. *T. kodakarensis* DAD was separately transformed by a series of *deaD* promoter variants, and the resulting transformants were grown in ASW-YT-S° medium at 60°C and 85°C. Cells were harvested and disrupted by sonication. Cells extracts were separated on 12% SDS-PAGE and transferred to PVDF membrane. *Pc*-Kat expression was investigated by western blotting analysis. The thermodynamic nearest-neighbor parameters was used to calculate the free energy change (-ΔG°) from the nucleotide sequence.

[Result and Discussion] Cold inducibility was confirmed when the region (five-adenosine (AAAAA) repeat) was replaced with AAAAAA sequence. Several plasmids containing AA, AAA, and AAAA were constructed and the results suggested that the reduction of adenosine number between the SD region and ATG is still functional for cold induction. To examine an effect of location of adenosine cluster, the five-adenosine sequence was transferred to the region before SD, showing that the cold inducibility was not observed. In order to investigate relation between the binding stability of mRNA and template DNA, the free energy change were calculated from the nucleotide sequence. The value of free energy change of each plasmid depends on the sequence between the SD and ATG. Repeated A or T cluster with free energy change of 50kcal/mol to 65 kcal/mol is required to achieve the cold induction. These data suggest that both binding stability and the nucleotide sequence are involved in cold induction.