Gene Regulation by Transcriptional Factors in Enterohemorrhagic *Escherichia coli* O157:H7

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a major food-borne infectious pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. We have reported the complete chromosome sequence of an O157:H7 strain isolated from the Sakai outbreak, and the results of genomic comparison with a laboratory strain K-12. The O157:H7 chromosome is 5.5Mb in size, 859 Kb larger than that of K-12. We identified a 4.1-Mb sequence highly conserved between the two strains, which may represent the fundamental backbone of the *E. coli* chromosome. The remaining 1.4-Mb sequence comprises of O157:H7-specific sequences, most of which are horizontally transferred foreign DNAs. The predominant roles of bacteriophages in the emergence of O157:H7 is evident by the presence of 24 prophages and prophage-like elements that occupy more than half of the O157:H7-specific sequences. The O157:H7 chromosome encodes 1632 proteins and 20 tRNAs that are not present in K-12. Among these, at least 131 proteins are assumed to have virulence-related functions.

Comparative study of transcriptome based on DNA microarray technology is very useful. However, expression of genes is generally regulated by multiple promoters, in most cases, controlled by specific transcriptional factors. DNA microarrays on the market can't detect such individual transcriptional units. DNA microarray data also contain both direct and indirect transcriptional regulation data. To solve these problems, we constructed a promoter cloning system that consists of a low copy number plasmid carrying the promoterless *lacZ* gene as a reporter gene and various *lacZ*-deletion strains desired for specific transcriptional factors. Using these methods, we isolated various transcriptonal factor (CRP, Lrp, AsnC, YbaO, PhoB, PhoP, Ecs0418, and Ecs5067)-dependent promoters. We will describe in focus on the isolation of CRP-dependent promoters from the *E. coli* O157 gemome.

Cyclic AMP receptor protein (CRP) is known to be one of the global transcription factors in *E. coli*. The activity of CRP is triggered by binding to cAMP in response to glucose levels in culture medium and other stresses. To identify promoters directly regulated by CRP-cAMP from the enterohemorrhagic *E. coli* O157:H7 genome, we constructed the cloning system. The about 700 bp DNA fragments obtained from the whole genome were randomly cloned into the plasmid containing the promoterless *lacZ* gene as a reporter gene. The *E.*

coli O157 whole genome shotgun bank was introduced into a *lac, cya*-deletion strain and incubated on LB plates. Colonies on the plates were replicated on LB/Xgal and LB/Xgal/cAMP plates, and the expressions of *lacZ* were compared by the colony color differences. For identifying promoters directly regulated by CRP-cAMP, we performed the electrophoretic mobility shift assay using CRP-cAMP complex and DNA fragments. Furthermore, for the purpose of identifying CRP-cAMP binding sites within the promoter region, we performed footprinting assays. After DNA sequencing, we identified 12 previously known and 30 novel CRP-cAMP dependent promoters in regions common to the K12 genome. Of the novel promoters, 3 internal promoters and 2 antisense promoters were included. We also identified 10 novel CRP-cAMP dependent promoters in O157 genome regions and 5 of them were antisense promoters. In the CRP-cAMP dependent promoters identified in our study, there were many promoters of the genes encoding for metabolic enzymes, molecular transporters, transcriptional regulators in K12 genome regions and promoters of the genes encoding for a two-component regulator, the prophage carrying of the *stx1* operon encoding Shiga toxin 1, an ATP dependent DNA helicase, etc. in O157 specific genome regions.

Furthermore, using similar methods, it is revealed that Lrp (leucine-responsive regulatory protein) represses expression from the promoter of the *stx1* operon, PhoB activates expression from the promoter of the operon encoding a type I secretion system and RTX toxin, and PhoP activates expression of the gene encoding type 1 fimbrial protein precursor in the *E. coli* O157:H7 specific regions.