

Doctoral Thesis (Abridged)

Epigenetic regulation by DNA methyltransferases in *Helicobacter pylori*

(ピロリ菌でのDNAメチル化酵素によるエピジェネティック制御)

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Summary

In bacteria, epigenetic DNA methylation plays an important role in regulation of gene expression, genome replication, cell cycle regulation, and genome maintenance. DNA methyltransferases are responsible for DNA methylation and most of them have high sequence specificity. Many of these methyltransferases are a member of a restriction-modification (RM) system and are called modification (M) enzymes. We hypothesized that, by generating diverse epigenomes, they contribute to adaptive phenotypes through influences on gene expression pattern. *Helicobacter pylori*, a human gastric pathogen responsible for most of gastric cancer incidence, shows high genetic diversity and methylome variability, which likely contribute to their lifelong persistence adapting to its host. As a result, it provides a great opportunity to test our hypothesis. My collaborators and I together knocked out several of its specificity determinant genes and examined transcriptome and phenotype for this purpose.

(1) Role of Type III restriction-modification systems of strain P12.

Here three Type III Mod genes were knocked out from strain P12 to examine their effects on methylome, transcriptome and phenotype. Comparative methylome analysis at the single base resolution by a Pacbio sequencer demonstrated that, a methyltransferase M.HpyPX is responsible for GACC methylation while the other two genes are found inactive in methylation. Type III Mod gene *hpyPXM* knockout showed large effects on transcriptome. Many flagellar genes associated with cell motility were repressed in the knockout mutant. Decreased expression in sets of genes associated with homologous recombination and nucleotide repair was observed. These suggested decrease in motility and sensitivity to DNA damaging agents. In phenotypic experiments, this mutant indeed completely lost motility and showed increased sensitivity to a DNA damaging agent. It also showed decrease cell viability under high acidic condition. However, the knock out mutation promoted growth during log and stationary phases. These results suggest that, Type III restriction-modification system HpyPX has a global influence on gene expression and controls phenotypes other than restriction-modification.

(2) Role of Type II DNA methyltransferases in strain P12.

Roles of DNA methyltransferases of four Type II RM systems in transcriptome and phenotypes were analyzed. Unique gene expression pattern was found in each knockout mutant. In $\Delta hpyPVIIM$, 53 genes were found differentially expressed. Those include *flaA*, encoding a flagellar protein, oxidative stress genes and other restriction-modification genes. As expected, this mutant showed higher motility in soft agar. This mutant also showed increased resistance to oxidative stress and acid stress. Gene restoration experiments indicated that these phenotypes were indeed linked with deleted M gene. About two thirds of all the genes were differentially expressed in another Type II M gene mutant $\Delta hpyPIM$. These two strains showed significantly impaired growth. In the other two mutants, no or only few genes were affected.

(3) Role of a Type I specificity gene, Type II and Type III methyltransferase genes in strain 26695.

Three genes responsible for target sequence specificity of DNA methyltransferases, a Type I specificity gene, a Type IIP M gene, and a Type III Mod gene, were knocked out in strain 26695. Unique gene expression pattern was found in each. For the knockout of a conserved Type IIP methyltransferase M.HpyAX, 151 genes were found differentially expressed, which included genes for outer membrane proteins, virulence and stress responses. Surprisingly, we found that DNA methylation-associated genes are overrepresented in the differentially expressed genes. This mutant is found to be sensitive to high acidic condition, which strongly suggested importance of the methyltransferase for survival of *H. pylori* under the extreme acidic condition in the stomach. On the contrary, in the other two knockouts of less conserved Type IS and Type III Mod genes, only a few genes are found to be differentially expressed which included the genes for an outer membrane protein related to host cell interaction. I also found that each of the mutants showed unique growth pattern during stationary phase. These results suggest that, through gene expression regulation, a complex network involving methyltransferase contributes to adaptive phenotype in *H. pylori*.

From these results, it is clear that not all but some DNA methyltransferase genes in a genome contribute to generation of a specific gene expression pattern and influence the phenotype in their unique way. This suggests that DNA methylation greatly contributes to biology of *H. pylori*. We found opposite effects of different methyltransferases on motility, oxidative stress, acid stress and growth. Therefore, RM systems prove to be important system for the regulation of gene expressions and maintain a balance condition in their host for survival under diverse condition. We also found some MTases affect expression of other MTases. The RM systems may maintain a complex regulatory network.