

Cucumber mosaic virus 2b protein alters the plant epigenome

キュウリモザイクウイルス 2b タンパク質発現 *Arabidopsis* における

エピジェネティクス解析

生物資源科学専攻 植物育種科学講座 植物病原学 外川 靖子

1. Introduction

Cucumber mosaic virus (CMV) has a wide range of plant hosts and causes major economic loss. The CMV 2b protein (2b) is multifunctional and important in plant-virus interaction; it is best known as an RNA silencing suppressor (RSS), which counteracts the host defense. 2b also influences the RNA-directed DNA methylation (RdDM) pathway, suggesting that 2b may induce epigenetic alterations at a genome-wide scale in plants. In the current study, I aim to elucidate genome-wide DNA methylation changes induced by CMV infection or solely by 2b.

2. Materials and Methods

Transgenic *Arabidopsis thaliana* lines (ecotype Col-0) that express 2b from CMV Y strain (CMV-Y) were prepared and named as Col-2b. I examined genome-wide DNA methylation in Col-2b by methylated DNA immunoprecipitation sequencing (MeDIP-Seq). Genome-wide DNA methylation was also tested in CMV-Y-infected *Arabidopsis* by whole-genome bisulfite sequencing (WGBS). To analyze the gene expression affected by 2b, I conducted real time PCR in Col-2b. I also performed RNA sequencing (RNA-Seq) and small RNA sequencing (sRNA-Seq) in Col-2b to examine the genome-wide gene expression changes.

3. Results

I confirmed the previous result that the histone 3 lysine 9 demethylase gene, *Increase in BONSAI Methylation 1 (IBM1)*, and DNA demethylase gene, *Repressor of Silencing 1 (ROS1)*, were downregulated in Col-2b. Downregulation of these genes was observed in lines that express 2b at a low level. Therefore, 2b may enhance both histone methylation and DNA methylation independently when it is expressed at a low level. 2b was shown to directly bind to the *IBM1* promoter region by chromatin immuno-precipitation, suggesting that 2b blocked *IBM1* transcription by the interaction. Bisulfite sequencing showed that the *ROS1* promoter region was less methylated in Col-2b than in WT, which agrees with the *ROS1* downregulation. MeDIP-Seq revealed that Col-2b exhibited hypermethylation at a genome-wide scale, but especially at the pericentromeric region, compared with WT. Consistently, WGBS showed that CMV-Y-infected plants were more hypermethylated than WT. Stress-responsive genes, such as *Jacalin* gene and *WRKY* genes, showed differential expression in Col-2b, in the manner that the plants become hypersensitive to pathogens. In addition, sRNA-Seq revealed that most miRNAs were downregulated in Col-2b, suggesting that the miRNA-target genes may be upregulated by 2b.

4. Conclusion

Here, I demonstrated that both CMV-Y infection and 2b alone induced genome-wide DNA hypermethylation in *Arabidopsis* perhaps via downregulation of *IBM1* and *ROS1*. Consequently, the expression of stress-responsive genes was altered in favor of CMV. I propose that CMV utilizes 2b to change the expression of key epigenetic regulators and “hijacks” the plant epigenome to compromise plant immunity. This proposal provides an additional layer of counter-defense mechanism by 2b.