学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称： 博　士（農学）　　氏名　中西　登志紀

学 位 論 文 題 名

Elucidation of the upstream gene regulatory network to
activate the Polyhedrin promoter in Bombyx mori nucleopolyhedrovirus

（カイコ核多角体ウイルスのポリヘドリンプロモーター活性化上流遺伝子の
制御ネットワークの解明）

Gene expression in Bombyx mori nucleopolyhedrovirus (BmNPV) is dynamically regulated throughout the course of infection. The BmNPV genome encodes 143 genes, with temporally characterized expression patterns. Notably, during the very late infection phase, the structural protein polyhedrin is expressed at extremely high levels. However, the system-level regulation activating the polyhedrin promoter remains poorly understood. The first chapter of this thesis focuses on developing techniques for the temporal and quantitative analysis of gene expression. The second chapter describes network modeling of the upstream gene regulatory network required for activating the polyhedrin promoter.

1. Clinically approved chemical-controlled suppression of protein expression in BmN Cells

Genetic perturbations are essential for elucidating the regulatory relationships between genes. Small molecule-controlled protein expression is beneficial for immediate inhibition, which is crucial for unveiling the changing regulatory relationships between baculovirus genes during viral infection progression. In this study, I utilized small molecule-assisted shut-off (SMASh), which controls protein abundance based on chemical triggers, to determine its effectiveness in BmN cells. Enhanced green fluorescent protein (EGFP) served as the target gene for SMASh. The results demonstrated that SMASh was effective in BmN cells, and the signal from the EGFP fused with a PEST sequence (a reference degron-like sequence in SMASh) was sufficient for fluorescence observation.

2. Elucidation of the regulatory system for polyhedrin promoter activity based of promoter activation profiles

To understand the activation of the polyhedrin promoter during the very late stage of BmNPV infection, it is necessary to comprehend the transition in the gene expression regulatory system as the infection progressed. This study involved constructing ten dual-reporter viruses, each containing the polyhedrin promoter and one of the ten essential gene promoters upstream of two different fluorescent proteins. The activity of the two promoters in each infected gene could be measured using a newly developed time-lapse observation system. Additionally, knockdown experiments of the ten essential genes were conducted, enabling analysis of the regulatory networks among essential genes at each infection stage and the impact of perturbing essential genes on polyhedrin promoter activity. This analysis identified *lef-8, lef-9*, and *lef-10* as marker genes for polyhedrin promoter activity and suggested the existence of at least two regulatory systems. It was also suggested that *p143* is closely associated with the

junction of these regulatory systems. Furthermore, screening experiments to identify genes affecting *p143* promoter activity from the entire gene set of BmNPV suggested one gene as being associated with the DNA replication system.