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## FUNCTIONAL ANALYSIS OF POLYCOMB GROUP PROTEINS IN THE SILKWORM, BOMBYX MORI

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SILKWORM, *BOMBYX MORI*  
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## 論 文 内 容 の 要 旨

Polycomb group (PcG) proteins are involved in chromatin modifications for maintaining gene repression that play important roles in the regulation of cell cycle progression, tumorigenesis, chromosome X-inactivation, and genomic imprinting in *D. melanogaster*, mammals, and even plants. Although the regulatory mechanisms of PcG complexes have been well understood right now, the emerging evidence have shown much more complicated and varied ways in different species. Therefore, the aim of this work is studied to understand the action mechanism of PcG system in the model insect *B. mori*.

In Chapter I, based on the updated silkworm genome sequence, a genome-wide identification of the *B. mori* PcG genes were performed by using the *D. melanogaster* PcG genes as queries. As a result, 13 PcG genes were identified in the silkworm genome. Comparison of the *B. mori* PcG proteins with those from other insect species was also analyzed and revealed a conserved evolution of the insect PcG proteins. In order to determine that the identified *B. mori* PcG genes act as PcG functions, several PcG genes with the complete open reading frame (ORF) were isolated and their subcellular localization in the silkworm cells as well as the transcriptional repression on the report system were also investigated. Moreover, knocking down of the PRC2 components *BmE(Z)*, *BmESC*, and *BmSU(Z)12* by RNA interference (RNAi), considerably decreased the global levels of H3K27me3, indicating the contribution of the *B. mori* PRC2 complex on the tri-methylation of H3K27.

In order to characterize the potential target genes regulated by the *B. mori* PcG proteins, in Chapter II, the genome-wide expression screening after RNAi of four PcG genes *BmSCE*, *BmESC*, *BmPHO*, and *BmSCM*, representing distinct complexes were carried out in the silkworm cells. The analysis for the up-regulated genes has revealed that the candidate target genes possess binding activity, catalytic activity, or transcription regulator activity, suggesting that *B. mori* PcG may play important roles in a variety of developmental processes via the regulation of its target genes.

And finally, to understand the regulatory mechanism of PcG proteins in a given genome locus, in Chapter III, a specific target gene *asparagine synthetase (BmASNS)* identified from microarray data in Chapter II was used to further study. The present result has elucidated a novel epigenetic regulation in which PcG complexes regulate *BmASNS* expression involving H3K27me3. The data demonstrated that PcG proteins repress the transcription of *BmASNS* gene through recruiting to the putative YY1 binding motifs and CpG island within the *BmASNS* promoter. It is therefore tempting to speculate that the YY1 binding element, as well

as the CpG island is sufficient to recruit PcG complexes and subsequently deposited H3K27me3 to repress target gene expression in *B. mori*. Particularly, this study provides important new insights into the mechanism underlying the dynamic regulation of its target gene by PcG system during the cell cycle.

In conclusion, the present study on the identification and functional characterization of *B. mori* PcG complexes has revealed some novel insights into the regulation mechanisms. These findings will also provide fundamental knowledge useful for further investigations of PcG functions in *B. mori*.