（様式第３号）

学 位 論 文 要 旨

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題目: Regulation of gene expression in cultured insect cells using *piggyBac* transposon
（*piggyBac*トランスポゾンを用いた昆虫培養細胞における遺伝子発現制御）

A number of cell lines have been established from various insect species and used for gene expression and protein production. Among these insect cell-based gene expression systems, the baculovirus expression vector system (BEVS) using lepidopteran host insect cells is most frequently used for protein production. In BEVS, however, several drawbacks including malfunction of cellular processes such as protein folding and secretion occur due to the virus infection, limiting its performance. These limitations can be overcome by using stably transformed insect cells, in which genes of interest are integrated in the genome and continuously or inductively expressed without virus infection. A lepidopteran insect-derived transposon, *piggyBac*, has been successfully used for transgenesis in various insects and other organisms including human cells. However, due to a relatively random fashion of *piggyBac* integration into the genome, its insertion sites and copy numbers among transformants considerably vary, resulting in significant differences in the expression level of transgene. For transformed insect cells, such differences in the transgene expression and their underlying mechanisms have never been studied, in spite of importance for developing novel technologies which enable highly efficient protein production in transformed insect cells. Thus, in this study, *piggyBac*-mediated transformation was performed using lepidopteran insect cells, BmN4, derived from the ovary of the silkworm, *Bombyx mori*, whose whole genome had been sequenced, and regulatory mechanisms of the transgene expression were investigated as follows.

First, to improve the conventional protocol for the *piggyBac*-mediated transformation by shortening the period of transposase production in the transformed BmN4 cells, the helper plasmid was replaced with the *in vitro* synthesized transposase mRNA, whose 5’ and 3’ UTR sequences except for the polyA tail were similar to those of the mature transcript expressed from the helper

plasmid. The mRNA was effective in the transposition of the *piggyBac* element from the co-transfected donor plasmid into the genome and was comparable to the helper plasmid in the efficiency of its transformation. In addition, the transfected mRNA disappeared earlier than the transcript from the helper plasmid and the transposase activity became undetectable after around 21 days of post-transfection during the antibiotic selection. The results clearly indicate that, as a source of transposase for the *piggyBac*-mediated transformation of BmN4 cells, the *in vitro* synthesized mRNA is superior in the stability of the integrated *piggyBac* element to the helper plasmid.

Next, to explore regulatory mechanisms of the transgene expression, BmN4 were transformed by a *piggyBac* vector harboring the green fluorescent protein (GFP) transgene as a marker and subjected to cloning by limiting dilution, resulted in the establishment of clonal transgenic cell lines with different levels of GFP expression including several lines with no detectable expression, although the *piggyBac*-mediated transgene integration in the genome DNA was confirmed in all of the lines. Some of the GFP-negative lines produced massively amplified GFP-derived PIWI-interacting RNAs (piRNAs) capable of silencing GFP *in trans*. In these lines where GFP expression was stably silenced by the piRNA pathway, a common transcript from an endogenous piRNA cluster, in which a part of the cluster was uniquely fused with an antisense GFP sequence. Bioinformatic analyses suggested that the fusion transcript was a source of GFP primary piRNAs. The results implicate a role for transcription from a piRNA cluster in initiating *de novo* piRNA production against a new insertion.

Finally, to develop a new technology for permanent knockdown of a specific endogenous gene based on the newly found mechanism of piRNA-mediated transgene silencing, BmN4 cells were transformed by *piggyBac* vectors harboring cDNAs of *B. mori* genes encoding methoprene toletant homolog 1 and 2 (BmMet1 and BmMet2) with a tandemly-arranged GFP cDNA as transgenes, and several clonal transgenic cell lines were established for each transgene. In some of the lines where GFP expression was stably silenced and antisense transcripts from BmMet1 or BmMet2 transgenes were detected, their sense transcripts were not detected. The results support the possibility that endogenous genes for BmMet1 and BmMet2 are silenced by piRNA-mediated pathway in the transformed cell lines, although further analyses are required to confirm it.

Overall, in this study, transgenic BmN4 cell lines with a wide range of transgene expression levels, from overexpression to complete supression, were successfully cloned by the improvement of *piggyBac*-mediated transformation protocol, and could be effectively used to reveal a part of mechanisms of the transgene silencing by piRNA pathway, which is expected to be applied for developing a novel RNA interference technology to control endogenous gene expression.