

Molecular Approach for the Insect Biotechnology

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In this presentation, I'd like to introduce the current research situation of insect biotechnology processing in my laboratory and examine the possible strategy to utilize insect functions and insects as genetic resources. Recent advances in insect molecular biology have highlighted the prospects of insect gene utility for improvement of insect biotechnology. Target genes that have been cloned for this purpose include those encoding for two insecticidal toxins, chitinase, defender against cell death and fibroin from the spider, *Araneus ventricosus*, toxin and luciferase from the firefly, *Pyrocoelia rufa*, and cellulase, ferritin, cuticle, storage protein and protease from the mulberry longicorn beetle, *Apriona germari*, etc.

Silkworm larvae offer a number of advantages over other insect larvae, such as larvae size, ease of handling, nonallergenic to human, and well-characterized genetics. For these reasons, silkworm is deeply being studied for the development of transgenic insect as a biofactory. In this study, gene targeting vector using fibroin gene and supersilkworm vector using ecdysteroid UDP-glucosyltransferase (*egt*) gene are constructed. Gene targeting vector has been demonstrated to be a gene targeting on the silkworm fibroin gene for transgenesis of silkworm producing hybrid silk. The result revealed that the targeted genes remained stable until at least F3 generations. The F1 silkworm introduced with supersilkworm vector sustained 1 or 2 days during the 5th larval stage, and cocoon weight is increased approximately 10% compared to control.

The complete sequence of the mitochondrial genome of the firefly, *P. rufa*, was determined. The circular genome is 17,736 bp long and contains a standard gene complement, i. e. thirteen genes encoding mitochondrial proteins, twenty-two transfer RNA (tRNA) genes, the large and small ribosomal RNA subunits, and the (A+T)-rich region. The gene order and arrangement was identical to that reported for other insects. In addition, the complete nucleotide sequence and the exon-intron structure of the luciferase gene of the *Hotaria*-group fireflies, *H. unmunzana*, *H. papariensis* and *H. tsushimana*. The luciferase gene of the *Hotaria*-group firefly including the known *H. parvula* spans 1,950 bp and consisted of six introns and seven exons coding for 548 amino acid residues, suggesting highly conserved structure among *Hotaria*-group fireflies. In conclusion, these results suggest that *H. unmunzana*, *H. papariensis* and *H. tsushimana* are very closed or they might be identical species at least based on the luciferase and COI genes.