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Application of Recombinant DNA Technology to Analysis of Gene Functions in Silkworm

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An application of recombinant DNA technology to silkworm genes has been initiated by purification and characterization of fibroin mRNA (Suzuki and Browon ,1972). Then, the fibroin gene was cloned (Oshima and Suzuki, 1977) and its gene regulation system has been studied using *in vitro* transcription in cell-free extracts. The main targets at early stage of the technology were genes related to the synthesis of silk in the silk gland. So far, genes for major components of silk proteins, sericine, fibroin H and L chains, fibrohexamarine (P25) were cloned, analyzed their structures and expression mechanisms. The other genes studied extensively are chorion consisting the egg shells. The egg shells are formed by regular lamellar structure and the components of the shell are mainly chorion proteins. The cohorin genes forms multi-gene family and occupied the region of more than 1000 kb on the second chromosome. It has been studied as a model to understand the function of developmentally regulated genes.

Then, the recombinant DNA technology has been applied to many other genes whose products have been studied biochemically. To clone the genes interested, cDNA library was screened by antibody or synthesized nucleotides. Alternative method to clone the interested genes were PCR using the primers designed nucleotide sequences from conserved nucleotide sequences in the interested genes in other organisms. By using these methods, many genes have been cloned and analyzed their structures and functions. However, as long as using these methods, numbers of genes cloned and analyzed are limited. To know the whole structure and interaction of genes in the silkworm genome, the progress of genome analysis and tools to understand the gene functions are indispensable.

Concerning to the genome analysis, EST data base of silkworm have been constructed and opened. Everyone can access the data base and use it although the coverage of EST is still about 30% and continued the effort to increase the independent EST in the data base. For the full sequencing of silkworm genome, whose size is 530 Mb, the BAC libraries were constructed and the works for making its contigs are under going. Furthermore, DNA micro array is already

constructed.

A development of system for gene function is another important problem. For this the germ–line transformation using transposable element *piggyBac* have been developed recently (Tamura et al., 2000) and used routinely in the experiments to study the function of genes interest and produce useful materials in the sillk gland. The system now move to gene silencing using RNAi, heterogous gene expression system using yeast UAS-GAL4.

We expect that the silkworm can be used in new fields of science by applying the new technology and develop new industry related to insects.