# UTILIZATION OF INSECT GENOME INFORMATICS FOR METABOLIC ENGINEERING OF INSECT CELLS

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For production of recombinant proteins with desired qualities by using insect gene expression systems such as baculovirus expression vector (BEV) and *Drosophila* S2 cell systems, we should carefully chose host insect cell lines with adequate posttranslational modification properties, because these properties are varied among cell lines. Until recent, pathways responsible to these properties have been characterized and compared by biochemical analyses of product structures and related enzymatic activities.

One of most intensively analyzed pathways of insect cells is the N-glycosylation pathway. In general, the insect N-glycosylation pathways are quite similar to the mammalian pathways until removal of mannose residues by mannosidase II as well as addition of fucose residues by fucosyltransferases. However, they differ in the last steps where removal of N-acetylglucosamine and mannose residues by Golgi-associated N-acetylglucosaminidase and mannosidases occur in the insect cells, while addition of N-acetylglucosamine residue by N-acetylglucosaminyltransferase II and further extension by addition of galactose and sialic acid residues occur in the mammalian cells (Altmann *et al.*, 1999).

Recently, we have developed a novel BEV system using the *Antheraea pernyi* nucleopolyhedrovirus (AnpeNPV) (Wang *et al.*, 2000, Kobayashi *et al.*, 2001) and demonstrated that productivities of diapausing pupae of both *A. pernyi* and *Samia cynthia pryeri* are higher than BEV systems using *Autographa californica* NPV (AcNPV) and *Bombyx mori* NPV (BmNPV) (Huang *et al.*, 2001, Kobayashi, 2001). The proteolytic degradation of recombinant protein occurred at later stage of infection was avoided by disrupting the functional cathepsin gene in the AnpeNPV genome (Huang *et al.*, 2002, Kobayashi, 2001). All of our results clearly demonstrated that diapausing pupae are excellent natural bioreactors for the recombinant protein production. Users do not have to have insect rearing facilities but just purchase the pupae and store in refrigerator until injecting recombinant viruses for the protein production. Virus infection and protein harvest are very easy and can be performed by automatic injectors and processors. In addition, there is a report that posttranslational modification such as a-amidation, which never occurs in cell culture, occurred in larvae and more efficiently in pupae (Hellers and Steiner, 1992).

We have evaluated N-glycosylation property of *A. pernyi* cells (NISES-AnPe-428; AnPe) by analyzing N-linked oligosaccharide added on recombinant insect peptide hormone, prothoracicotropic hormone, PTTH, which has one N-glycosylaion site in each subunit of homodimer. By lectin blot analysis, we occasionally obtained results indicating a relatively high potential for the complex type N-glycan formation in AnPe cells. Further characterization of N-glycans have demonstrated that the proportion of N-acetylglucosamine terminated N-glycans was

significantly higher than AcNPV/Sf9 cell system, supporting the complex-type N-glycan formation potential of AnPe cells. However, the efficiency of complex N-glycan formation in *A. pernyi* cells is quite low and insufficient for practical production of pharmaceutical glycoproteins.

Recent progresses in genome projects of *Drosophila* (Adams *et al.*, 2000) and *Bombyx* have made possible the analysis of the expression levels of multiple genes simultaneously using DNA microarrays. This novel and powerful technology is quite useful to comprehend host insect cell physiology from gene expression profile and to evaluate effects of genetic manipulations to improve posttranslational modification properties. Therefore, it seemed to be much better to improve the N-glycosylation property as well as other posttranslational modifications of lepidopteran cell lines by metabolic engineering than by searching and evaluating new cell lines, because abundant and complete genome informatics will enable us to accomplish the altering metabolic pathways of insect cells rationally and efficiently. We are just going to start the analysis of two *B. mori* cell lines, BmN4 and NISES-BoMo-15AIIc, using DNA microarrays containing 10,000 unique ESTs to reveal the cell-line dependent differences in the gene expression profile.

We also decided to use *Drosophila* S2 cell as a model system for metabolic engineering of insect N-glycosylation pathway. For S2 cells, foreign gene are easily introduced and expressed by transformation using cotransfection with a drug resistant gene plasmid or infection of recombinant baculovirus at very high titer (Culp *et al.*, 1991; Lee *et al.*, 2000). Gene silencing using RNA interference is technically well established (Hammond *et al.*, 2000). It may be possible to activate useful genes in the genome which are inactivated in the cell line but active certain tissues at specific developmental stage of *Drosophila* by artificial expression of limited number of master genes. We have already established transformed *Drosophila* S2 cells expressing PTTH under control of hsp70 promoter and found the similar N-glycosylation pattern to those of lepidopteran cells used in BEV systems. Using this S2 cell system, we will establish methods for metabolic engineering of N-glycosylation pathway, and then transfer the techniques to lepidopteran cells. The same strategy can be also applicable for metabolic engineering of any other posttranslational modification pathways.

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