The Production of Heterologous Proteins Using the Baculovirus Expression Vector System in Insect Cells

Tae Won Goo*, Tae-Young Kwon**, Sang-Han Lee*** and O-Yu Kwon[†]

Department of Anatomy, College of Medicine, Chungnam National University, Taejon 301-747, Korea
* Department of sericulture and Entomology, National Science and Technology, RDA, Suwon 441-100, Korea
** Cheongdo Peach Experiment Station, Kyoungbuk Provincial Agricultural Technology Administration, Kyoungbuk 714-850, Korea
*** Korea Research Institute of Bioscience & Biotechnology, Yusung-ku, Taejon 305-333, Korea

Abstract The baculovirus expression vector system (BEVS) is one of the powerful heterologous protein expression systems using insect cells. As a result this has become a hot issue in the field of biotechnology. The advantage of the BEVS is that the large-scale production of heterologous proteins, which undergo posttranslational modification in the endoplasmic reticulum (ER), can be accomplished. Although posttranslational modification of heterologous proteins in insect cells is more similar to mammalian cells than yeast, it is not always identical. Therefore, aggregation and degradation can sometimes occur in the ER. To produce a high level of bioactive heterologous proteins using BEVS in insect cells, the prerequisite is to completely understand the posttranslational conditions that determine how newly synthesized polypeptides are folded and assembling with ER chaperones In the ER lumen. Here, we provide Information on current BEVS problems and the possibility of successful heterologous protein production from mammalian cells.

Key words: Baculovirus expression vector system (BEVS), Insect cells, Posttranslational modification, Endoplasmic reticulum (ER), ER chaperones, Heterologous protein

Introduction

In eukaryotic cells, in order to secret proteins from the endoplasmic reticulum (ER) to the extracellular space, the following four steps are essential [2]: 1) the formation of a mRNA/ribosome complex on the ER membrane and starting translation from the mRNA to the nascent polypeptide; 2) the posttranslational modification in the ER via glycosylation, phosphorylation, myristylation, folding and assembly; 3) a concentration of secretory proteins in the Golgi apparatus and a recycling of the proteins associated with transport from the ER to the Golgi complex; 4) selection of the secretory vesicle to the outside space of the plasma membrane. Of these four steps, ER posttranslational modification is the rate-limiting step in the export of newly synthesized secretory proteins from a cell. In fact, the destination of almost all secretory and plasma membrane proteins depend on whether it can be escape freely from the ER, after correct folding and/or assembly in the ER lumen. If this step is not perfect, malfolded proteins are trapped within ER and this may impede cell physiology [2,24].

Currently, the requirement of heterologous proteins produced from insect culture cells for diagnostics, protein studies, biomedical studies and vaccines has increased [35]. For these reasons, the stable production of heterologous proteins has been a major focus biotechnology. However, proteins made from insect cells using the baculovirus expression vector system (BEVS) sometimes exhibit low bioactivity and low secretion including protein aggregation and degradation in the ER lumen [15,24]. To produce many highly processed heterologous proteins from insect culture cells, it is important to understand fully the function of the ER during posttranslational modification of the secretory proteins [23,27]. Here we report the processing of the ER posttranslational modification of secretory protein in insect cells by BEVS, including some current problems, and possible solutions to these problems In addition, the production of heterologous proteins from mammalian culture cells by BEVS is also briefly described

Signal peptide processing

The eukaryotic secretory pathway of both plasma membrane proteins and the secretory proteins is a complex multi-organelle system. Each of these contains specific proteins that assist in the secretion process. The first step of protein synthesis in the eukaryotic cells is mRNA translation from the ribosomes bound to the ER and the nascent polypeptides

*Corresponding author
Phone: 82-42-580-8206, Fax: 82-42-586-4880
E mail: oykwon@cnu.ac.kr

across the ER membrane. In this time, a cytosolic hsp70 chaperone [18] assists the translocation process and suppresses polypeptide aggregation with the hsp40 [37], Hip [20] and Hop [29] cofactors. It was demonstrated that these cofactors are necessary for hsp70 functional activity. The result of the coexpression experiment on human hsp70 and murine immunoglobulin G (IgG) in insect cells using BEVS, revealed the secretion of highly active IgG[1]. Recently an interesting experiment was attempted to determine a more effective method of translation. This method involves the replacement of the signal peptide from heterologous protein, which is well recognized, and is easily cleaved by the signal peptidase [41]. Indeed, another recent study demonstrated that replacing the prepropapain signal peptide with melittin's enhanced propapain secretion. Although the detail mechanism is relatively known, the useful modification of the signal peptide should improve the ability to obtain a heterologous protein from the insect culture cells using BEVS. However, although a similar modification of the signal peptide was successful in obtaining amount of the heterologus protein it could not guarantee a perfectly bioactive protein that can act as a native protein undergoing posttranslational modification in the lumen [3,28].

Posttranslational modification

The transformation of the one-dimensional genetic information (DNA-RNA) into the three-dimensional protein structures (protein) depends on the accuracy and efficiency of the process of protein folding. In addition, the maintenance of this correct conformation is essential for the protein functions in a cell. All eukaryotic cells secrete several kinds of specialized proteins for the plasma membrane and the secretory proteins via the ER lumen, where posttranlational modification, folding, oligomerization and assembly of the protein occur [14,26]. One of the most important functions of the ER is to provide an intracellular environment to facilitate the proper folding and assembly of newly synthesized exportable proteins that reside with numerous molecular chaperones, which determine the protein's final destination [17,32].

The term chaperone has been used in Europe since antiquity. A molecular chaperone in the biological field is a term used to describe proteins that prevent incorrect interactions between parts of other molecules. However, they do not impart steric information or form part of the final functional structures. Classically, a newly synthesized polypeptide was thought to attain its functional conformation with no assistance from other intracellular molecules and with no further expenditure of bioenergy. Creighton observed that in many cases protein denaturation is not fully reversible *in vitro*, especially at physiological temperatures and at the proper protein concentrations *in vivo* [6,7]. This proposal is supported by the growing number of instances where the proteins do not assemble correctly at the rates and yields required *in vivo* unless other preexisting proteins

are present to assist them. These types of pre-existing proteins in the cell are referred to as molecular chaperones. Ellis defined the molecular chaperone in 1987 as a family of unrelated classes of proteins that mediate the correct assembly of other polypeptides in a cell, in the cytosol and in the ER [10]. A molecular chaperone is defined as any protein that binds another protein and has no function of its own and it is not involved in the final bioactive product [11,14].

In response to physiological or environmental stress, the lumen of the ER provides a distinct folding environment where there is an oxidizing potential, and there is a relatively high concentration of Ca^{2+} [8,33]. This produces a distinct protein family called ER chaperones, which are ubiquitous, abundant, and well conserved through evolution among eukaryotic cells [14]. Two of the major ER chaperones are the Bip and GRP94, members of the Hsp70 and Hsp90 families, which are classified according to their size and sequence similarity [40]. Another and two are the Ca^{2+}-binding proteins, calnexin and calreticulin[17,30]. In ER, chaperones provide two important functions in protein folding. The first is to catalyze the protein-folding reactions by the protein disulfide protein (PDI) and cis-trans prolyl isomerase [12,13]. The second is to prevent the protein-folding intermediates from self-aggregating and energetically stabilize unfavorable conformations by Bip and the glucose - regulated protein family (GRPs) [5,31,44].

BEVS has been used to produce a number of secretory proteins including a glutamate transporter, protein kinase, melanotransferrin, Factor X, interleukin 6 and an ion transport peptide [34]. However, insoluble aggregates or improperly posttranslated proteins frequently accumulated in the ER. This is one of the major limitations in producing effective heterologous proteins using BEVS in insect cells [2,23,27]. For effective secretion, processing, and glycosylation of several heterologous proteins in insect cells, the role of molecular chaperones in the ER needs to be fully understood. In this paper the results of successfully Improved experiments using chaperones that reside in the ER are introduced. When a recombinant Bip is coexpressed in insect cells with IgG, and there is increased solubility and secreted IgG levels obtained from the insect cells, PDI overexpression can also increase IgG folding and secretion from the Insect cell [2,22,25,36,45]. Moreover, calnexin and calreticulin overexpression can also assist the folding and assembly of heterologous proteins in insect cells[34,39], When using the BEVS to produce heterologous proteins, the biggest problem is oligosaccharide processing in the ER because glucose trimming enzymes and sugars are different between insect and human cells [9]. This can lead to differences in the structures of the final attached carbohydrates on the heterologous proteins [4,15]. Wagner et al recently reported that coexpressed GlcNAc transferase I increased the level of complete heterologous glycoproteins from insect

cells [42,43]. The production of siafylated recombinant glycoproteins in the BEVS will require metabolic engineering efforts to extend the native protein glycosylation pathways of insect cells [2].

To establish stable/useful heterologous expression in insect cells using the BEVS, how protein folding occurs in the ER associated with ER chaperones, especially how to perfect glycosylation occurs, needs to be understood. In addition, understanding the subtle mutations in the coding sequences of exportable proteins, in conjunction with ER quality control, can lead to defective protein trafficking, resulting in poor production of heterologous proteins. It will also prove essential to address the mechanistic and cell biological questions of the native protein conformations that are associated with ER chaperones.

BEVS in human cells

It has long been known that even though mammalian cells can take up baculoviruses, these viruses eventually fail to replicate using a host-cell's enzymes in a similar way as with other mammalian pathogenic viruses [16,38]. However, most recently, an interesting experiment demonstrated that recombinant baculoviruses containing a copy of luciferase reporter gene under the cytomegalovirus (CMV) promoter could be taken up by human hepatocytes and produce heterologous proteins within these cells [19]. Another study showed that about 25% of enhanced gene expression was induced using the Rous sarcoma virus promoter. Currently, there is no actual use for the direct expression of baculoviruses in mammalian cells, although there is some promise in using the BEVS in mammalian cells. Therefore, further study is recommended. The merit of the BEVS in mammalian cells for somatic gene therapy is that expressing exogenous proteins using baculoviruses may be safe because low levels of transcription do not have an adverse effect on the host mammalian cells[35]. Compared to using animal viruses, the baculovirus does not use intracellular factors that are unsuitable for direct expression since these are adopted through a different evolutionary process. Baculovirus expression in mammalian cells mainly depends on the modified baculovirus and associated intercellular factors. The BEVS in mammalian cells would be quite useful for somatic gene therapy in mammalian cells. In addition if a BEVS is used to induce to efficiently target certain types of mammalian cells, it may be able to induce apoptosis in cancer cells. Because baculovirus expression in mammalian cells is quite limited, it can be used to target a specific organ without side effects. Therefore, further study is needed to produce stable heterologous proteins using the BEVS in mammalian cells.

Acknowledgment

References

1. Ailor, E. and M. J. Betenbaugh. 1998. Overexpression of a cytosolic chaperone to improve solubility and secretion of a recombinant IgG protein in insect cells. Biotechnol. Bioeng. 58, 196-203.
2. Ailor, E. and M. J. Betenbaugh. 1999. Modifying secretion and post-translational processing in insect cells. Curr. Opin. Biotechnol. 10, 142-145.
3. Ailor, E., J. Pathmanathan, J. D. Jongbloed and M. J. Betenbaugh. 1999. A bacterial signal peptidase enhances processing of a recombinant single chain antibody fragment in insect cells. Biochem. Biophys. Res. Commun. 255, 444-450.
4. Altman, F. 1996. N-glycosylation in insects revisited. Trends Glycosci. Glycotech. 8, 101-114.
5. Craig, E. A. 1985. The heat shock response. CRC Crit. Rev. Biochem. 18, 239-280.
6. Creighton, T. E., D. A. Hillson and R. B. Freedman. 1980. Catalysis by protein-disulphide isomerase of the unfolding and refolding of proteins with disulphide bonds. J. Mol. Biol. 142, 43-62.
7. Creighton, T. E., A. Zapun and N. J. Darby. 1995. Mechanisms and catalysts of disulfide bond formation in proteins. Trends Biotechnol. 13, 18-23.
8. Darby, N. J., J. Kemmink and T. E. Creighton. 1996. Identifying and characterizing a structural domain of protein disulfide isomerase. Biochemistry 35, 10517-10528.
9. Davies, A. H. 1995. Advances in the use of recombinant baculoviruses for the expression of heterologous protein. Curr. Opin. Biotechnol. 6, 543-547.
10. Ellis, R. J. 1987. Proteins as molecular chaperones. Nature 328, 378-379.
11. Ellis, R. J. 1993. The general concept of molecular chaperones. Phil. Trans. Roy. Soc. Lnd. B. 339, 257-261.
12. Fischer, G., E. Berger and H. Bang. 1989. Kinetic beta-deuterium isotope effects suggest a covalent mechanism for the protein folding enzyme peptidylprolyl cis/trans-isomerase. FEBS Lett. 250, 267-270.
13. Freedman, R. B., T. R. Hirst and M. F. Tuite. 1994. Protein disulphide isomerase: building bridges in protein folding. Trends Biochem. Sci. 19, 331-336.
14. Gething, M. J. and J. Sambrook. 1992. Protein folding in the cell. Nature 355, 33-45.
15. Goochee, C. F., M. J. Gramer, D. C. Andersen, J. B. Bahr and J. R. Rasmussen. 1991. The oligosaccharides of glycoproteins: bioprocess factors affecting oligosaccharide structure and their effect on glycoprotein properties. Biotechnology 9, 1347-1355.
16. Groner, A., R. R. Granados and J. P. Burand. 1984. Interaction of Autographa californica nuclear polyhedrosis virus with two nonpermissive cell lines. Intervirology 21, 203-209.
17. Hammond, C. and A. Helenius. 1994. Quality control in the secretory pathway: retention of a misfolded viral membrane glycoprotein involves cycling between the ER, intermediate compartment, and Golgi apparatus. J. Cell Biol. 126, 41-52.
18. Hartl, F. U. and J. Martin. 1995. Molecular chaperones in cellular protein folding. Curr. Opin. Struct. Biol. 5, 92-102.
19. Hofmann, C., V. Sandig, G. Jennings, M. Rudolph, P. Schlag and M. Strauss. 1995. Efficient gene transfer into human hepatocytes by baculovirus vectors. Proc. Natl. Acad. Sci.

USA **92**, 10099-10103.

20. Hohfeld, J., Y. Minami and F. U. Hartl. 1995. Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. *Cell* **83**, 589-598.

21. Hollister, J. R., J. H. Shaper and D. L. Jarvis. 1998. Stable expression of mammalian beta 1,4-galactosyltransferase extends the N-glycosylation pathway in insect cells. *Glycobiology* **8**, 473-480.

22. Hsu, T. A. and M. J. Betenbaugh. 1997. Coexpression of molecular chaperone BiP improves immunoglobulin solubility and IgG secretion from *Trichoplusiani* insect cells. *Biotechnol. Prog.* **13**, 96-104.

23. Hsu, T. A., J. J. Eiden and M. J. Betenbaugh. 1994. Engineering the assembly pathway of the baculovirus-insect cell expression system. *Ann. N. Y. Acad. Sci.* **721**, 208-217.

24. Hsu, T. A., N. Takahashi, Y. Tsukamoto, K. Kato, I. Shimada, K. Masuda, E. M. Whiteley, J. Q. Fan, Y. C. Lee and M. J. Betenbaugh. 1997. Differential N-glycan patterns of secreted and intracellular IgG produced in *Trichoplusia ni* cells. *J. Biol. Chem.* **272**, 9062-9070.

25. Hsu, T. A., S. Watson, J. J. Eiden and M. J. Betenbaugh. 1996. Rescue of immunoglobulins from insolubility is facilitated by PDI in the baculovirus expression system. *Protein Expr. Purif.* **7**, 281-288.

26. Hurtley, S. M. and A. Helenius. 1989. Protein oligomerization in the ER. *Ann. Res. Cell Biol.* **5**, 277-307.

27. Jarvis, D. L. and A. Garcia. 1994. Biosynthesis and processing of the *Autographa californica* nuclear polyhedrosis virus gp64 protein. *Virology* **205**, 300-313.

28. Jarvis, D. L., M. D. Summers, A. Garcia and D. A. Bohlmeyer. 1993. Influence of different signal peptides and prosequences on expression and secretion of human tissue plasminogen activator in the baculovirus system. *J. Biol. Chem.* **268**, 16754-16762.

29. Johnson, B. D., R. J. Schumacher, E. D. Ross and D. O. Toft. 1998. Hop modulates Hsp70/Hsp90 interactions in protein folding. *J. Biol. Chem.* **273**, 3679-3686.

30. Kim, P. S. and P. Arvan. 1995. Calnexin and BiP act as sequential molecular chaperones during thyroglobulin folding in the endoplasmic reticulum. *J. Cell Biol.* **128**, 29-38.

31. Kirkpatrick, R. B., S. Ganguly, M. Angelichio, S. Griego, A. Shatzman, C. Silverman and M. Rosenberg. 1995. Heavy chain dimers as well as complete antibodies are efficiently formed and secreted from *Drosophila* via a BiP-mediated pathway. *J. Biol. Chem.* **270**, 19800-19805.

32. Knittler, M. R., S. Dirks and I. G. Haas. 1995. Molecular chaperones involved in protein degradation in the endoplasmic reticulum: quantitative interaction of the heat shock cognate protein BiP with partially folded immunoglobulin light chains that are degraded in the endoplasmic reticulum.

Proc. Natl. Acad. Sci. USA **92**, 1764-1768.

33. Macer, D. R. and G. L. Koch. 1988. Identification of a set of calcium-binding proteins in reticuloplasm, the luminal content of the endoplasmic reticulum. *J. Cell Sci.* **91**, 61-70.

34. Pfeifer, T. A. 1998. Expression of heterologous proteins in stable insect cell culture. *Curr. Opin. Biotechnol.* **9**, 518-521.

35. Possee, R. D. 1997. Baculoviruses as expression vectors. *Curr. Opin. Biotechnol.* **8**, 569-572.

36. Ritchie, P. J., A. Decout, J. Amey, C. J. Mann, J. Read, M. Rosseneu, J. Scott and C. C. Shoulders. 1999. Baculovirus expression and biochemical characterization of the human microsomal triglyceride transfer protein. *Biochem. J.* **338**, 305-310.

37. Sugito, K., M. Yamane, H. Hattori, Y. Hayashi, I. Tohnai, M. Ueda, N. Tsuchida and K. Ohtsuka. 1995. Interaction between hsp70 and hsp40, eukaryotic homologues of DnaK and DnaJ, in human cells expressing mutant-type p53. *FEBS Lett.* **358**, 161-164.

38. Tjia, S. T., G. M. zu Altenschildesche and W. Doerfler. 1983. *Autographa californica* nuclear polyhedrosis virus (AcNPV) DNA does not persist in mass cultures of mammalian cells. *Virology* **125**, 107-117.

39. Vassilakos, A., M. F. Cohen-Doyle, P. A. Peterson, M. R. Jackson and D. B. Williams. 1996. The molecular chaperone calnexin facilitates folding and assembly of class I histocompatibility molecules. *EMBO J.* **15**, 495-506.

40. Vogel, J. P., L. M. Misra and M. D. Rose. 1990. Loss of BiP/GRP78 function blocks translocation of secretory proteins in yeast. *J. Cell Biol.* **110**, 1885-1895.

41. von Heijne, G. 1994. Signals for protein targeting into and across membranes. *Subcell. Biochem.* **22**, 1-19.

42. Wagner, R., H. Geyer, R. Geyer and H. D. Klenk. 1996a. N-acetyl-beta-glucosaminidase accounts for differences in glycosylation of influenza virus hemagglutinin expressed in insect cells from a baculovirus vector. *J. Virol.* **70**, 4103-4109.

43. Wagner, R., S. Liedtke, E. Kretzschmar, H. Geyer, R. Geyer and H. D. Klenk. 1996b. Elongation of the N-glycans of fowl plague virus hemagglutinin expressed in *Spodoptera frugiperda* (Sf9) cells by coexpression of human beta 1,2-N-acetylglucosaminyltransferase I. *Glycobiology* **6**, 165-175.

44. Welch, W. J., H. S. Kang, R. P. Beckmann and L. A. Mizzen. 1991. Response of mammalian cells to metabolic stress; changes in cell physiology and structure/function of stress proteins. *Curr. Top. Microbiol. Immunol.* **167**, 31-55.

45. Whiteley, E. M., T. A. Hsu and M. J. Betenbaugh. 1997. Thioredoxin domain non-equivalence and anti-chaperone activity of protein disulfide isomerase mutants *in vivo*. *J. Biol. Chem.* **272**, 22556-225563.