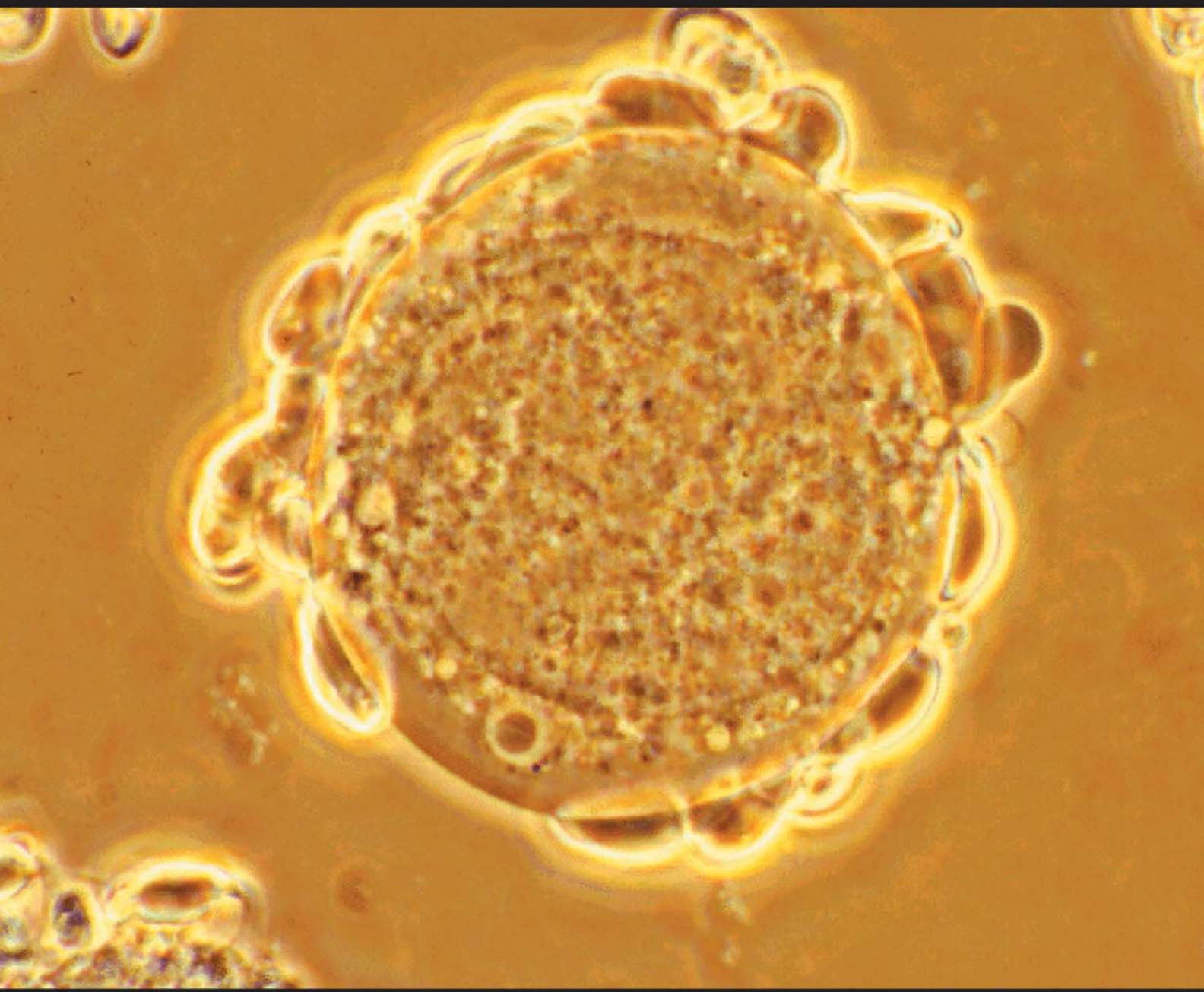
A Publication of
The Williamsburg BioProcessing Foundation

January/February 2003
ISSN 1538-8786

BioProcessing JOURNAL

Advances & Trends In Biological Product Development



Vol. 2/No. 1

www.bioprocessingjournal.com

Development of Novel Transgenic Insect Cell Lines that Support Humanized Glycoprotein Production by Baculovirus Expression Vectors

DONALD L. JARVIS, JASON R. HOLLISTER, AND JARED J. AUMILLER

The baculovirus-insect cell system consists of a recombinant baculovirus vector and its host, which may be a lepidopteran insect larvae or an established lepidopteran insect cell line.[1,2,3] Hundreds of different recombinant proteins have been produced using the baculovirus-insect cell system, facilitating biomedical research on protein structure, function, and the roles of various proteins in disease. In addition, many biotechnology companies are using this system to produce recombinant proteins for potential clinical use as vaccines, therapeutics, or diagnostic reagents. Protein Sciences uses the baculovirus-insect cell system exclusively to provide recombinant proteins for human clinical trials, and to routinely produce, under cGMP conditions, the massive, infected insect cell cultures needed to meet those needs. Onyx Pharmaceuticals has reported that they intend to use the baculovirus-insect cell system to express every known human protein for proteome chips that will be used for high-throughput functional screens. Dendreon Corporation has produced a therapeutic cancer vaccine that promises to be one of the first products of the baculovirus-insect cell system to be approved for a human clinical application. Thus, the baculovirus-insect cell system is well-established as an important bioprocessing tool for a variety of different applications.

The major advantages of the baculovirus-insect cell system include its ability to produce large amounts of recombinant protein, its relatively low cost, the absence of adventitious agents infectious for humans, and its ability to provide eucaryotic protein modifications such as glycosylation. Glycosylation is a common covalent chemical modification that can directly, or indirectly, influence protein function.[4,5,6,7] Sophisticated pathways are responsible for protein glycosylation in higher eucaryotic cells, such as mammalian cells, and these pathways are well understood.[8,9] In contrast, relatively few studies have focused on the nature of protein glycosylation pathways in insect cells. For nearly 15 years, one of the major research goals in our lab has been to elucidate and modify protein glycosylation pathways, with a focus on protein *N*-glycosylation in insect cell systems. Thus, the remainder of this article will focus on the nature of the insect cell *N*-glycosylation pathway and our continuing efforts to create transgenic insect cell lines with humanized *N*-glycosylation pathways. We expect these cell lines to be widely useful for the production of more authentic recombinant glycoproteins by baculovirus expression vectors.

Studies on the *N*-glycan processing enzymes and *N*-glycan structures produced by mosquito cells provided an early view of the protein N-glycosylation pathway in insect cells.[10] These studies indicated that the insect *N*-glycan processing pathway is truncated relative to the mammalian pathway. That is, the insect processing pathway appeared to include the glycosidases involved in *N*-glycan trimming, but not all of the glycosyltransferases involved in *N*-glycan elongation (Fig. 1). Subsequent structural studies of the *N*-glycans isolated from the recombinant glycoproteins produced by baculovirus-infected lepidopteran insect cells, together with the apparent absence of sialyltransferase activity and CMP-sialic acid in these cells, generally supported this conclusion.[11] Accordingly, it is now widely believed that the major processed *N*-glycans on insect cell-derived glycoproteins are paucimannose structures (Man_3-$GlcNAc_2$-*N*-Asn) either with or without fucose residues linked to the chitobiose (GlcNAc-GlcNAc-*N*-Asn) core. Some data also indicate that insects have the potential for more extensive *N*-glycan processing, which allows them to produce complex, even terminally sialylated, *N*-glycans under some condi-

Corresponding author ***Donald L. Jarvis, Ph.D.****, is a Professor, Department of Molecular Biology; DLJarvis@uwyo.edu.* ***Jason R. Hollister, Ph.D.*** *and* ***Jared J. Aumiller****, a research associate, are with the Department of Molecular Biology, University of Wyoming, Laramie, Wyoming.*

tions.[12-21] However, this capability is quite limited and clearly does not reflect the major *N*-glycan processing pathway in the baculovirus-insect cell system.

Therefore, in the final analysis, baculovirus-infected lepidopteran insect cells typically fail to produce complex, terminally sialylated *N*-glycans like those produced by mammalian cells. This result poses a serious problem for those interested in using the baculovirus-insect cell system for recombinant glycoprotein production. In particular, the absence of terminal sialic acids on the recombinant glycoproteins produced with this system is problematic because glycoproteins lacking sialic acids have extremely short half lives *in vivo*.[5,7,22] The inability of the baculovirus-insect cell system to produce recombinant glycoproteins with authentic glycan side chains is one of its most significant limitations, and has inhibited the use of this system for recombinant glycoprotein production.

To address this problem, our group began using mammalian glycosyltransferase genes to create transgenic insect cell lines with extended N-glycan processing capabilities. By the late 1980's, we had successfully adapted technology from mammalian cell systems to establish methods for stable genetic transformation of lepidopteran insect cell lines.[23] Later, we created a series of improved expression plasmids for insect cell transformation using a promoter from an immediate early baculovirus gene, *ie1*, which provides constitutive foreign gene expression in uninfected lepidopteran insect cell lines; plus a baculovirus enhancer element, *hr5*, which stimulates *ie1*-mediated transcription.[24] Finally, we began to insert cDNAs encoding mammalian N-glycan processing enzymes (generously provided by the glycobiology community) into these expression plasmids. The resulting constructs were used together with various selectable markers to transform two commonly used lepidopteran insect

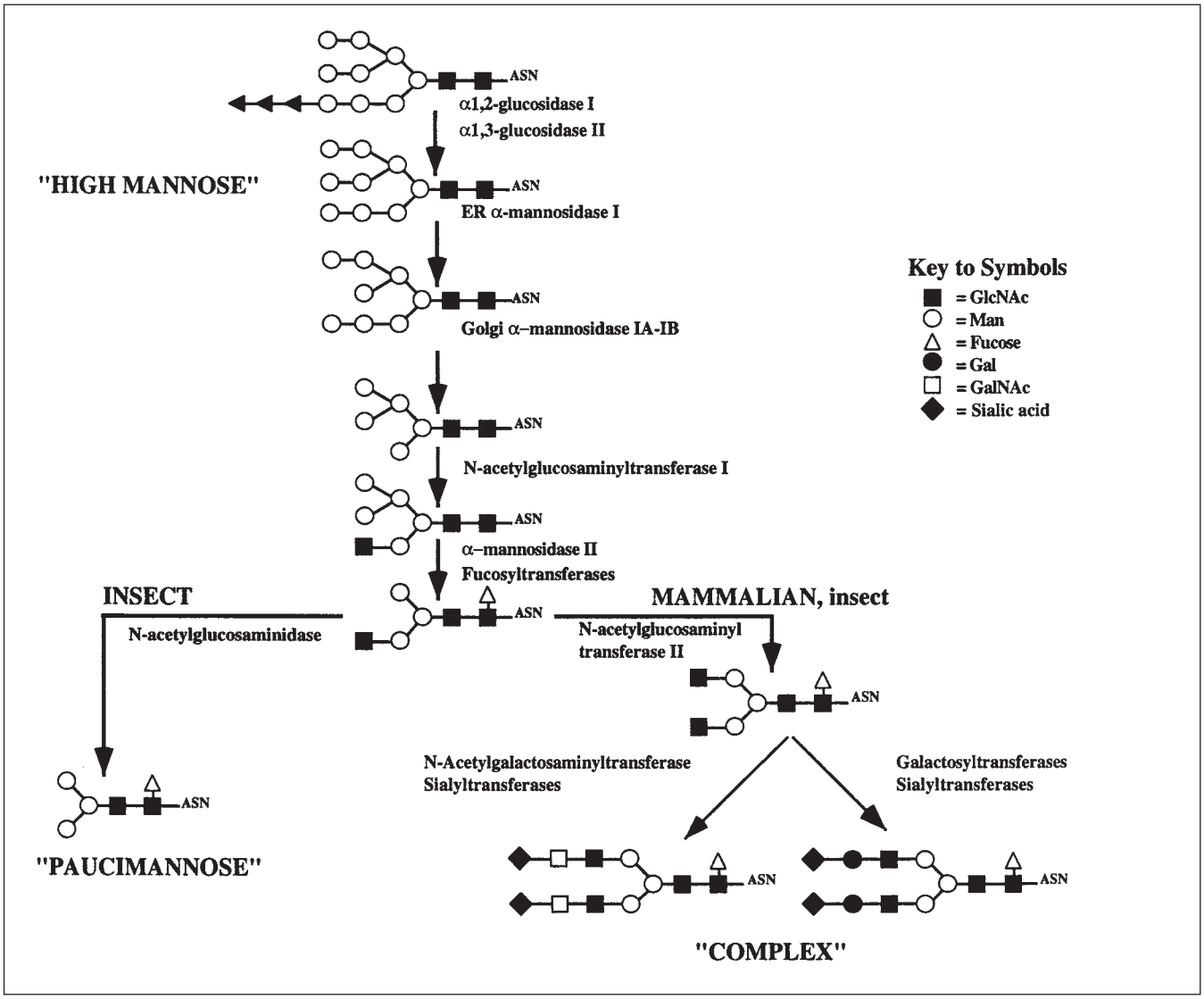


Figure 1. An overview of protein *N*-glycosylation pathways in insect and mammalian cells. Monosaccharides are indicated by their standard symbolic representations, which are defined in the key. The insect and mammalian *N*-glycan processing pathways share a common intermediate, as shown. The major products derived from this intermediate are paucimannose *N*-glycans in insect cells and complex *N*-glycans in mammalian cells. Complex *N*-glycans are broadly defined and far more diverse than indicated in the Figure, which includes only representative examples of biantennary, terminally sialylated structures.

cell lines, Sf9 and Tn-5B1-4 (High Five™).[25,26]

The first insect cell line stably transformed in this fashion was Sfß4GalT, which were Sf9 cells with stably integrated copies of a mammalian ß1,4-galactosyltransferase (ß4GalT) gene in their genomes.[27] This new cell line had normal growth properties, supported baculovirus infection, and both contained and constitutively expressed the integrated ß4GalT gene under *hr5-ie1* control. In addition, expression of this gene induced ß4GalT activity in the transgenic cells, which allowed Sfß4GalT cells, unlike the parental Sf9 cells, to produce terminally galactosylated foreign glycoproteins during baculovirus infection. Subsequently, both Sf9 and Tn-5B1-4 cells were doubly-transformed with the ß4GalT gene, plus a mammalian α2,6-sialyltransferase gene, which yielded the first transgenic lines to express both mammalian enzymes, designated Sfß4GalT/ST6 and Tn5ß4GalT/ST6.[28,29] Using lectin blotting and HPLC analyses, we demonstrated that these cell lines, unlike their progenitors, could produce recombinant glycoproteins with terminally sialylated *N*-glycans during baculovirus infection.

Detailed mass spectroscopic analyses of the *N*-glycans produced by Sfß4GalT/ST6 cells revealed, however, that they were monoantennary structures in which only the lower (α3) branch had been elongated. From a metabolic engineering perspective, this result was unacceptable because native mammalian glycoproteins have no monoantennary *N*-glycans. A simple inspection of the mammalian *N*-glycosylation pathway (Fig. 1) suggested that the reason these cell lines failed to produce biantennary *N*-glycans was that they had inadequate levels of *N*-acetylglucosaminyltransferase-II (GlcNAc-TII) activity, which is the enzyme responsible for initiating elongation of the upper (α6) branch.[30] This hypothesis was supported by a previous study which showed that cell lines derived from *Spodoptera frugiperda,* and other insects, have extremely low levels of endogenous GlcNAc-TII activity.[31]

We recently tested this hypothesis by genetically transforming Sf9 cells with expression plasmids that encode five mammalian glycosyltransferases including ß4GalT, ST6GalI, and GlcNAc-TII, to produce a transgenic insect cell line designated as SfSWT-1.[32] Like their progenitors, SfSWT-1 cells had normal growth properties and supported baculovirus infection. These cells also constitutively produced RNAs from all five glycosyltransferase genes and contained high levels of ß4GalT, ST6GalI, and GlcNAc-TII activities. An attempt has not been made at this time to measure the activities encoded by the two other mammalian transgenes, *N*-acetylglucosaminyltransferase I and α2,3-sialyltransferase, expressed by these cells. We have, however, used a recombinant baculovirus to produce a glutathione-S-transferase-tagged form of a *Spodoptera frugiperda* Golgi α-mannosidase-I (GST-SfManI) in SfSWT-1 cells.[33] This GST-tagged product is routinely used as a model glycoprotein in our lab because it has only one *N*-glycan that can be completely removed, and the protein can be highly expressed and quickly purified to apparent homogeneity. The GST-SfManI isolated from SfSWT-1, and several other insect cell lines, were affinity purified on a glutathione-Sepharose column, and the *N*-glycans were removed from the protein, harvested, and analyzed by HPLC and mass

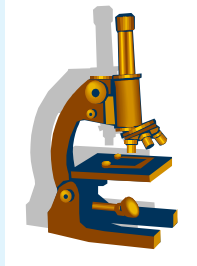
Cell Culture Media Development

-Experience in cell culture media development for optimizing cell number or protein yield.
-Modern, well equipped cell biology laboratory including flow cytometry, image analysis and biochemistry.
-Examples of successful projects include:

*Developed several serum-free cell culture media for commercial use.
*Several fetal bovine serum substitutes sold commercially.
*Media component development.
*Novel protein combinations containing extracellular matrices and attachment factors.

-Have employed advanced computer and multivariant analysis in media design.
-GLP/ISO quality documentation and the ability to develop your project from concept through pilot scale with sales and marketing integration.
-Confidential and cost effective partnership.

For More Information and InquiriesContact:
Anthony R. Torres, M.D.
Immunogenetics Laboratory/CPD
Utah State University
(435) 797-2750
email: cellmedia@yahoo.com



spectroscopy. The results of these structural analyses clearly demonstrated that the recombinant glycoprotein produced by SfSWT-1 cells had acquired biantennary, terminally monosialylated structures at an efficiency of about 50%. The non-sialylated N-glycan subpopulation on this protein consisted primarily of biantennary, terminally digalactosylated structures. In addition, we obtained the same qualitative results upon analysis of the *N*-glycans that were isolated from a recombinant human glycoprotein expressed in SfSWT-1 cells, although the sialylation efficiency was lower. Thus, these results have demonstrated that induction of GlcNAc-TII, ß4GalT, and ST6GalI activities allows baculovirus-infected Sf9 cells to produce recombinant glycoproteins with humanized *N*-glycans.

The biantennary, terminally monosialylated structure produced by SfSWT-1 cells is the most extensively processed *N*-glycan produced by any transgenic insect cell line described to date. The reason for the absence of terminal sialic acid on the upper (α6) branch of these N-glycans has not been determined. Considering, however, that the upper branch is preferentially sialylated by α2,3-sialyltransferases, and not ST6GalI, the most obvious possibility is that SfSWT-1 cells have no α2,3-sialyltransferase activity.[34] Considering that the mammalian α2,3-sialyltransferase gene is transcribed, and the resulting RNA accumulates in SfSWT-1 cells, this enzyme might be unstable, mislocalized, or perhaps requires a cofactor that is absent in Sf9 cells. Studies are in progress to distinguish among these possibilities.

In a parallel collaboration with the Lee and Betenbaugh labs at Johns Hopkins University, we also have shown that higher levels of GlcNAc-TII activity are necessary for biantennary *N*-glycan production in Tn5ß4GalT cells.[35] In this study, we engineered a novel baculovirus expression vector to encode human GlcNAc-TII under *hr5-ie1* control, and human transferrin under polyhedrin control. The purpose of this experimental approach was to induce GlcNAc-TII activity in these cells during the early phases of infection, such that functional levels of both GlcNAc-TII and ß4GalT would be available for transferrin processing. The transferrin would be produced later in infection, under the control of the polyhedrin promoter. Three-dimensional HPLC analyses of the *N*-glycans isolated from transferrin, which was produced in Tn5ß4GalT cells using baculovirus vectors with and without the GlcNAc-TII gene, showed that only the former acquired biantennary *N*-glycans. This study demonstrated that Tn-5B1-4 cells also have inadequate levels of GlcNAc-TII to support routine production of humanized recombinant glycoproteins.

Considering that Sf9 cells have no detectable CMP-sialic acid, which is the donor substrate required by mammalian sialyltransferases, the ability of our transgenic insect cell lines to produce sialylated *N*-glycans was quite surprising.[36,37] These new cell lines had been engineered to produce mammalian glycosyltransferases, but we had taken no steps to enable them to produce or transport CMP-sialic acid. A compelling question arose from these observations: how can transgenic insect cells, which have no obvious source of CMP-sialic acid, sialylate newly synthesized glycoproteins? Clearly, one requirement is the intracellular sialyltransferase activity encoded by a mammalian transgene in these cells, because Sfß4GalT cells, which were transformed solely with the mammalian ß4GalT gene, produce no sialylated *N*-glycans on any recombinant glycoprotein that we have examined. In addition, recent studies in our lab have revealed that Sfß4GalT/ST6 and SfSWT-1 cells, both of which encode and express a mammalian sialyltransferase, can produce sialylated recombinant glycoproteins only when they are cultured in a growth medium containing fetal bovine serum (J.R. Hollister and D.L. Jarvis, submitted for publication). Interestingly, serum-free media supplemented with serum, that was dialyzed with a 50,000 molecular weight cutoff membrane, also supported sialoglycoprotein production by these cell lines. This result eliminated the rather remote possibility that low molecular weight compounds, such as CMP-sialic acid precursors, were the factors in serum supporting glycoprotein sialylation by these cells. Furthermore, serum-free media supplemented with a purified mammalian sialoglycoprotein, but not its desialylated counterpart, supported sialoglycoprotein production by these cells. Together, these results define the minimal requirements for sialoglycoprotein production by transgenic lepidopteran insect cell lines as (i) intracellular sialyltransferase activity and (ii) an exogenous sialoglycoprotein. Our working hypothesis is that these cells can scavenge terminal sialic acid from extracellular sialoglycoproteins and somehow process it into a form that can be utilized by the intracellular sialyltransferase.

One of our current goals is to investigate this model in much greater detail. Discovering the exogenous sialoglycoprotein requirement revealed that our transgenic insect cell lines could not produce recombinant sialoglycoproteins when cultured in serum-free media. This condition is a problem because the use of a serum-containing growth medium would raise obvious safety and regulatory issues in the industry, and would complicate efforts to recover and purify any recombinant glycoprotein produced by these cell lines. In addition, if this mode of obtaining sialic acid is inefficient, the putative scavenging mechanism might limit the efficiency of recombinant glycoprotein sialylation by SfSWT-1 cells. It is clear, therefore, that future metabolic engineering efforts in this system should be directed toward the production of transgenic lepidopteran insect cell lines, that can express mammalian enzymes which produce CMP-sialic acid from a sialic acid precursor, as well as the mammalian glycosyltransferases. In a previous study, conventional baculovirus vectors were used to express human sialic acid phosphate synthase and CMP-sialic acid synthetase genes under polyhedrin control in Sf9 cells.[38] Co-expression of these two enzymes in cells cultured in Ex-Cell 405 medium (JRH Biosciences, Lenexa, KS), and supplemented with N-acetylmannosamine, resulted in the production of a large intracellular pool of CMP-sialic acid. These results indicate that combining

CMP-SAS and glycosyltransferase production in a single transgenic insect cell line by adding sialic acid phosphate synthase and CMP-sialic acid synthetase genes to SfSWT-1 cells would yield a new cell line that can produce sialylated recombinant glycoproteins when cultured in a serum-free medium.

In summary, the inability to produce authentic mammalian glycans has been one of the most significant limitations of the baculovirus-insect cell expression system. However, we have been able to successfully address this limitation by genetically transforming established lepidopteran insect cell lines with constitutively expressible mammalian genes. This approach has yielded transgenic insect cell lines which have normal growth properties, support baculovirus infection, have new N-glycan processing enzyme activities, and can produce humanized recombinant glycoproteins.

Acknowledgments

We gratefully acknowledge the National Institutes of Health (GM49734) and the National Science Foundation (BES-9814157 and BES-9818001) for supporting our work. We also thank Invitrogen Corporation for a travel grant in support of the mass spectroscopic analyses and HyClone, Inc. for providing their serum-free insect cell growth medium, Sfx-Insect.

References

1. Luckow, V. L. and Summers, M. D. (1988) Trends in the development of baculovirus expression vectors. *Bio/Technology* **6**, 47-55.
2. O'Reilly, D. R., Miller, L. K., and Luckow, V. A. (1992) *"Baculovirus expression vectors,"* W.H. Freeman and Company, New York.
3. Jarvis, D. L. (1997) Baculovirus expression vectors in *"The Baculoviruses"* (L. K. Miller, Ed.), pp. 389-431. Plenum Press, New York.
4. Varki, A. (1993) Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* **3**, 97-130.
5. Bhatia, P. K. and Mukhopadhyay, A. (1999) Protein glycosylation: implications for *in vivo* functions and therapeutic applications. *Adv. Biochem. Engr. Biotechnol.* **64**, 155-201.
6. Dwek, R. A. (1995) Glycobiology: Towards understanding the function of sugars. Biochem. *Soc. Trans.* **23**, 1-25.
7. Raju, T. S., Lerner, L., and O'Connor, J. V. (1996) Glycopinion: biological significance and methods for the analysis of complex carbohydrates of recombinant glycoproteins. *Biotechnol. Appl. Biochem.* **24**, 191-194.
8. Kornfeld, R. and Kornfeld, S. (1985) Assembly of asparagine-linked oligosaccharides. *Ann. Rev. Biochem.* **54**, 631-664.
9. Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., and Marth, J. (1999) *"Essentials of Glycobiology,"* Cold Spring Harbor Press, Cold Spring Harbor, New York.
10. Marz, L., Altmann, F., Staudacher, E., and Kubelka, V. (1995) Protein glycosylation in insects in *"Glycoproteins"* (J. Montreuil, Vliegenthart, J. F. G., and Schachter, H., Eds.), pp. 543-563. Elsevier, Amsterdam.
11. Marchal, I., Jarvis, D. L., Cacan, R., and Verbert, A. (2001) Glycoproteins from insect cells: sialylated or not? *Biol. Chem.* **382**, 151-159.
12. Ogonah, O. W., Freedman, R. B., Jenkins, N., Patel, K., and Rooney, B. (1996) Isolation and characterization of an insect cell line able to perform complex N-linked glycosylation on recombinant proteins. *Bio/Technology* **14**, 197-202.
13. Hsu, T. A., Takahashi, N., Tsukamoto, Y., Kato, K., Shimada, I., Masuda, K., Whiteley, E. M., Fan, J. Q., Lee, Y. C., and Betenbaugh, M. J. (1997) Differential N-glycan patterns of secreted and intracellular IgG produced in *Trichoplusia ni* cells. *J. Biol. Chem.* **272**, 9062-9070.
14. Rudd, P. M., Downing, A. K., Cadene, M., Harvey, D. J., Wormald, M. R., Weir, I., Dwek, R. A., Rifkin, D. B., and Gleizes, P. E. (2000) Hybrid and complex glycans are linked to the conserved N-glycosylation site of the third eight-cysteine domain of LTBP-1 in insect cells. *Biochemistry* **39**, 1596-1603.
15. Davidson, D. J., Fraser, M. J., and Castellino, F. J. (1990) Oligosaccharide processing in the expression of human plasminogen cDNA by lepidopteran insect (*Spodoptera frugiperda*) cells. *Biochemistry* **29**, 5584-5590.
16. Watanabe, S., Kokuho, T., Takahashi, H., Takahashi, M., Kubota, T., and Inumaru, S. (2001) Sialylation of N-glycans on the recombinant proteins expressed by a baculovirus-insect cell system under ß-N-acetylglucosaminidase inhibition. *J. Biol. Chem.* **277**, 5090-5093.
17. Joshi, L., Shuler, M. L., and Wood, H. A. (2001) Production of a sialylated N-linked glycoprotein in insect cells. *Biotech. Progr.* **17**, 822-827.
18. Roth, J., Kempf, A., Reuter, G., Schauer, R., and Gehring, W. (1992) Occurrence of sialic acids in Drosophila melanogaster. *Science* 256, 673-675.
19. Karacali, S., Kirmizigul, S., Deveci, R., Deveci, O., Onat, T., and Gurcu, B. (1997) Presence of sialic acid in prothoracic glands of *Galleria mellonella (Lepidoptera). Tissue and Cell* **29**, 315-321.
20. Karacali, S., Kirmizigul, S., and Deveci, R. (1999) Sialic acids in developing testis of *Galleria mellonella (Lepidoptera). Invert. Repr. Dev.* **35**, 225-229.
21. Kim, K., Lawrence, S. M., Park, J., Pitts, L., Vann, W. F., Betenbaugh, M. J., and Palter, K. B. (2002) Expression of a functional *Drosophila melanogaster* N-acetylneuraminic acid (Neu5Ac) phosphate synthase gene: Evidence for endogenous sialic acid biosynthetic ability in insects. *Glycobiology* **12**, 73-83.
22. Weigel, P. H. (1994) Galactosyl and N-acetylgalactosaminyl homeostasis: a function for mammalian asialoglycoprotein receptors. *Bioessays* **16**, 519-524.
23. Jarvis, D. L., Fleming, J. A., Kovacs, G. R., Summers, M. D., and Guarino, L. A. (1990) Use of early baculovirus promoters for continuous expression and efficient processing of foreign gene products in stably transformed lepidopteran cells. *Bio/Technology* **8**, 950-955.
24. Jarvis, D. L., Weinkauf, C., and Guarino, L. A. (1996) Immediate early baculovirus vectors for foreign gene expression in transformed or infected insect cells. *Prot. Expr. Pur.* **8**, 191-203.
25. Summers, M. D. and Smith, G. E. (1987) A manual of methods for baculovirus vectors and insect cell culture procedures. *Tx. Ag. Expt. Stn. Bull.* No. 1555.
26. Wickham, T. J., Davis, T., Granados, R. R., Shuler, M. L., and Wood, H. A. (1992) Screening of insect cell lines for the production of recombinant proteins and infectious virus in the baculovirus expression system. *Biotech. Progr.* **8**, 391-396.
27. Hollister, J. R., Shaper, J. H., and Jarvis, D. L. (1998) Stable expression of mammalian beta 1,4-galactosyltransferase extends the N-glycosylation pathway in insect cells. *Glycobiology* **8**, 473-480.
28. Hollister, J. and Jarvis, D. L. (2001) Engineering lepidopteran insect cells for sialoglycoprotein production by genetic transformation with mammalian ß1,4-galactosyltransferase and a2,6-sialyltransferase genes. *Glycobiology* **11**, 1-9.
29. Breitbach, K. and Jarvis, D. L. (2001) Improved glycosylation of a foreign protein by Tn-5B1-4 cells engineered to express mammalian glycosyltransferases. *Biotech. Bioengr.* **74**, 230-239.
30. Bendiak, B. and Schachter, H. (1987) Control of glycoprotein synthesis. Kinetic mechanism, substrate specificity, and inhibition characteristics of UDP-N-acetylglucosamine:alpha-D-mannoside beta 1-2 N-acetylglucosaminyltransferase II from rat liver. *J. Biol. Chem.* **262**, 5784-5790.
31. Altmann, F., Kornfeld, G., Dalik, T., Staudacher, E., and Glossl, J. (1993) Processing of asparagine-linked oligosaccharides in insect cells. *N*-acetylglucosaminyltransferase I and II activities in cultured lepidopteran cells. *Glycobiology* **3**, 619-625.
32. Hollister, J. R., Grabenhorst, E., Nimtz, M., Conradt, H. O., and Jarvis, D. L. (2002) Engineering the protein N-glycosylation pathway in insect cells for production of biantennary, complex N-glycans. *Biochemistry*, in press.
33. Kawar, Z., Herscovics, A., and Jarvis, D. L. (1997) Isolation and characterization of an alpha 1,2-mannosidase cDNA from the lepidopteran insect cell line Sf9. *Glycobiology* **7**, 433-443.
34. Grabenhorst, E., Hoffmann, A., Nimtz, M., Zettlmeissl, G., and Conradt, H. S. (1995) Construction of stable BHK-21 cells coexpressing human secretory glycoproteins and human Gal(beta 1-4)GlcNAc-R alpha 2,6-sialyltransferase: alpha 2,6-linked NeuAc is preferentially attached to the Gal(beta 1-4)GlcNAc(beta 1-2)Man(alpha 1-3)-branch of diantennary oligosaccharides from secreted recombinant beta-trace protein. *Eur. J. Biochem.* **232**, 718-725.
35. Tomiya, N., Howe, D., Aumiller, J. J., Pathak, M., Park, J., Palter, K., Jarvis, D. L., Betenbaugh, M. J., and Lee, Y. C. (2003) Complex-type biantennary N-glycans of recombinant human transferrin from Trichoplusia ni insect cells expressing mammalian ß1,4-galactosyltransferase and ß1,2-N-acetylglucosaminyltransferase II. *Glycobiology* in press.
36. Hooker, A. D., Green, N. H., Baines, A. J., Bull, A. T., Jenkins, N., Strange, P. G., and James, D. C. (1999) Constraints on the transport and glycosylation of recombinant IFN-gamma in Chinese hamster ovary and insect cells. *Biotech. Bioengr.* **63**, 559-572.
37. Tomiya, N., Ailor, E., Lawrence, S. M., Betenbaugh, M. J., and Lee, Y. C. (2001) Determination of nucleotides and sugar nucleotides involved in protein glycosylation by high-performance anion-exchange chromatography: sugar nucleotide contents in cultured insect cells and mammalian cells. *Analyt. Biochem.* **293**, 129-137.
38. Lawrence, S. M., Huddleston, K. A., Tomiya, N., Nguyen, N., Lee, Y. C., Vann, W. F., Coleman, T. A., and Betenbaugh, M. J. (2001) Cloning and expression of human sialic acid pathway genes to generate CMP-sialic acids in insect cells. *Glycoconj. J.* **18**, 205-213.