PATENTS AND LITERATURE

INTRODUCTION

The objective of the Patents and Literature Section is to summarize and cite recent developments in industrial and academic research as portrayed within the scope of current patents and literature and to highlight emerging biotechnological research areas. The subject of the current Patent and Literature Section is The Use of The Baculovirus Expression System for Producing Recombinant Proteins.

PATENTS

This section covers patents concerning the use of the baculovirus expression system for producing recombinant proteins from the period of January 1983 to May 1991. This time period was considered in order that the total history of the use and development of the baculovirus system, from its initial development in 1983 to the present, could be highlighted. The major search headings were baculovirus and insect cells. The major patents recovered under this search are described below. Many of the abstracts are edited for clarity. The following patents are listed below: European Patent Organization (EP), World Intellectual Property Organization (WO), and United Kingdom, (GB). Copies of US patents can be obtained from the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Acs, G. and Price, P.
INFECTION OF LIVE INSECTS WITH A RECOMBINANT BACULOVIRUS FOR MANUFACTURE OF HETEROLOGOUS PROTEIN
WO 9001556, February 22, 1990
Assignee: Mount Sinai School of Medicine

A method for producing high levels of foreign protein with live insects comprises (1) coinfecting an insect cell culture with a wild-type baculovirus and a recombinant baculovirus in which the polyhedrin coding sequence has been replaced by a foreign gene, (2) infecting insects with the viruses found in the cell culture medium from step (1), (3) harvesting and lysing the insects after the viruses have replicated in the insects, and (4) purifying the protein from the lysate. The hepatitis B virus surface antigen gene was inserted into plasmid pAc610. Recombinant *Autographa californica* nuclear polyhedrosis virus was produced by standard techniques, and *Spodoptera frugiperda* cells were co-infected with the recombinant and wild-type viruses at ratios of 10:1. After 5 days, the resulting supernatant was fed to *Trichoplusia ni* larvae. After 5 days, the yield of antigen was ~60 μg/100 mg insect, representing 1.5% of the total extracted protein. The antigen was produced in glycosylated and unglycosylated forms just as in hepatitis B virus-infected humans. The antigen was immunogenic in mice.

Bishop, D.H.L. and Polly, R.
PRODUCTION OF BLUETONGUE VIRUS ANTIGENS USING A BACULOVIRUS EXPRESSION VECTOR FOR USE AS VACCINES
EP 279,661 August 24, 1988
Assignee: Oxford Virology Ltd.

Antigenic proteins of bluetongue virus (BTV), which can be used as vaccines, were produced by culture of insect cells infected with a recombinant virus. Recombinant *Autographa californica* nuclear polyhedrosis virus containing the gene for BTV-10 VP2 protein or BTV-17 VP3 protein was prepared. *Spodoptera frugiperda* cells infected with these recombinant baculoviruses produced the VP2 and VP3 proteins. The recombinant VP2 induced antibodies in mice and rabbits

which r eutralized infections of BTV-10, and to a lesser extent BTV-11 and BTV-17, but not BTV-13. VP3-induced antibodies neutralized BTV-10 and BTV-17 infections.

Bishop, D.H.L. and Emery, V.C.
EXPRESSION VECTOR FOR SIMULTANEOUS MANUFACTURE OF PROTEINS IN INSECTS
EP 305,087 March 1, 1989
Assignee: Natural Environmental Research Council

A procedure for producing expression vectors for the simultaneous manufacture of many recombinant proteins in insects, e.g., *Spodoptera frugiperda*, was developed. Plasmid PAcVC2, containing two fragments in opposite orientation, was constructed. Plasmid PAcVC2 consists of (1) the lymphocytic choriomeningitis virus (LCMV) N gene 3052-nucleotide fragment containing the polyhedrin promoter and transcriptional initiation and termination sequences and (2) a 7.3 kb *Autographa californica* nuclear polyhedrosis virus (AcNPV) fragment containing the AcNPV polyhedrin gene and its regulatory sequence. Recombinant virus VC2 was produced by co-transfecting *S. frugiperda* with PAcVC2 and a polyhedrin-negative helper baculovirus. VC2 virus-infected *S. frugiperda* cells produced LCMV-N protein at a level comparable to that obtained from YM1.YN1 virus infected cells (where LCMV-N protein, but not AcNPV polyhedrin protein, was expressed). Likewise, VC2 virus-infected *S. frugiperda* cells produced polyhedrin protein at a comparable level to that obtained by wild-type baculovirus (where AcPNV polyhedrin protein, but not LCMV-N protein, was expressed).

Clark, R., Devlin, P.E., Piatak, M., and O'Rourke, E.C.
INSECT SIGNAL PEPTIDE-MEDIATED SECRETION OF RECOMBINANT PROTEINS
WO 9,005,783 May 31, 1990
Assignee: Cetus Corporation

Insect cell signal peptides are used in recombinant manufacturing of heterologous proteins in insect cells to enhance the secretion of the protein. Plasmids pLP19 and pPD36, encoding a eukaryotic and insect cell signal peptide, respectively, and a plasminogen activator fusion product were constructed and transfected in the *Spodoptera frugiperda* Sf9 insect cell line along with baculovirus DNA for *in vivo* packaging. Recombinant baculoviruses PD36 and LP19 were again transfected into Sf9 cells; the Sf9 cell transformants were cultured by standard methods for production and secretion of the plasminogen activator fusion product. In a casein/agarose assay for plasminogen activator activity, the PD36-infected cells secreted approximately 5 times the enzyme activity secreted by LP19-infected cells.

Cochran, M.A.
EXPRESSION OF SELECTED POLYPEPTIDE GENES IN INSECT CELLS USING RECOMBINANT BACULOVIRUS
EP 228,036 July 8, 1987
Assignee: Microgenesys, Inc.

An insertion vector pAcPn-10, containing an *Autographa californica* nuclear polyhedrosis virus (AcMNPV) gene promoter followed downstream by an unique *Bam*IHI site, was constructed. This vector was used to express the vaccinia virus growth factor (VGF) gene. The expression of the VGF gene was effected by (1) inserting the gene in the unique *Bam*HI site of pAcPn-10; (2) transferring the gene to the genome of *Spodoptera exempta* nuclear polyhedrosis-virus-25 (SeMNPV-25) by homologous recombination to yield pMC160; and (3) co-transfecting *Spodoptera frugiperda* cells with pMC160 and SeMNPV-25. A partially glycosylated 18 kdal biologically active VGF was produced.

Cochran, M.A., Smith, G.E., and Volvovitz, F.
PRODUCTION OF POLYPEPTIDES DERIVED FROM THE ENVELOPE GENE OF HUMAN IMMUNODEFICIENCY VIRUS IN RECOMBINANT BACULOVIRUS-INFECTED INSECT CELLS FOR USE AS VACCINE
EP 265,785 May 4, 1988
Assignee: Microgenesys, Inc.

AIDS virus antigenic proteins were produced using a recombinant insect virus. Recombinant plasmids were constructed by inserting the cDNA for the HIV full-length envelope gp160 glycoprotein (or fragment thereof from plasmid NA-2) into plasmids MGS-3, -4, or -5. In some of the recombinant plasmids, the viral glycoprotein cDNA was fused with the gene for hepatitis B surface antigen or interleukin 2 signal peptide. The recombinant plasmids were calcium phosphate-precipitated with *Autographa californica* nuclear polyhedrosis virus and added to *Spodoptera frugiperda* cells. Cells harboring recombinant viruses containing inserts of HIV *env* sequences were isolated, which produced the desired viral proteins.

Cochran, M.A., Smith, G.E., and Volvovitz, F.
METHOD FOR PRODUCING RECOMBINANT CIRCUMSPOROZOITE ANTIGEN OF PLASMODIUM FLACIPARUM
EP 329,257 August 23, 1989
Assignee: Microgenesys, Inc.

A method for recombinant manufacture of circumsporozoite antigen (CA) of *Plasmodium falciparum* in insect cell culture using a baculovirus vector system was developed. Two recombinant baculovirus vectors, p3691 and p4148, for expression of the CA gene of *P. falciparum* from the *Autographa californica* polyhedrosis virus (AcMNPV) polyhedrin promoter were constructed. The resultant recombinant baculovirus vectors were used to manufacture recombinant CA's whose properties closely resembled those of authentic CA.

Devauchelle, G., Cerutti, M., Crozier, G., and Croizier, L.
A MODIFIED BACULOVIRUS EXPRESSION VECTOR FOR SPODOPTERA FRUGIPERDA CELLS
EP 345,152 December 6, 1989
Assignee: Institut National de la Recherche Agronomique; Centre National de la Recherche Scientifique

The genome of the *Spodoptera frugiperda* nuclear polyhedrosis baculovirus (SFNPV) modified by removal of restriction sites was used as an expression vector using a strong late promoter from the virus (e.g., the polyhedrin promoter). This virus was used for the direct cloning of the sequence to be expressed rather than by *in vivo* recombination. The *Sma*I site on the *Pst*I fragment G of the SFNPV genome was removed by *in vivo* recombination between the modified fragment cloned in pAT153 and the virus. A *Sma*I site was then introduced adjacent to the ATG codon of the polyhedrin gene by the same procedure. Sequences encoding the acetylcholinesterase of *Drosophila melanogaster* and *Escherichia coli* β-galactosidase were cloned into this site. These products were produced upon infection of cells.

Estes, M.K.
THE SYNTHESIS AND IMMUNOGENICITY OF ROTAVIRUS GENES USING A BACULOVIRUS EXPRESSION SYSTEM
EP 273,366 July 6, 1988
Assignee: Baylor College of Medicine

Recombinant baculoviruses containing rotavirus protein genes were prepared and the proteins produced in virally-infected insect cells. These antigenic proteins are used for vaccination against rotavirus-caused diarrhea, and the antigens or antibodies to the antigens are used to diagnose infection with rotavirus. Recombinant *Autographa californica* nuclear polyhedrosis viruses containing simian rotavirus SA11 gene 6, 9, or 10 were prepared by standard methods. *Spodoptera frugiperda* cells infected with Sa11-6 (containing gene 6) produced protein VP6, which was purified by immunoaffinity chromatography. Antisera to VP6 produced in guinea pigs reacted with rotavirus strains representing each known human serotype and subgroup by both immunofluorescence and ELISA assays. Mouse dams were parenterally immunized with VP6. Pups born to these mice were partially protected from challenge with rotavirus.

Fraser, M.J., Rosen, E.D., and Ploplis, V.A.
RECOMBINANT VECTOR-HOST SYSTEM FOR FOREIGN GENE EXPRESSION FROM HELIOTHIS POLYHEDRIN PROMOTER
WO 8,807,087 September 22, 1988
Assignee: American Biogenetic Sciences, Inc.

The *Heliothis zea* nuclear polyhedrosis virus (HzNPV) polyhedrin gene was cloned and sequenced. Plasmid pHE2.61ac was constructed by fusing the *lacZ* gene of *E. coli* to 5' sequences of the *H. zea* polyhedrin gene. This plasmid was used for the *in vivo* construction of a recombinant HzNPV that produced β-galactosidase in *H. zea* larvae (0.1% total weight) and cultured cells (200 $\mu g/10^6$ cells).

Gheysen, D., and Jacobs, E.
PRODUCTION OF RETROVIRAL GAG PROTEINS IN EUKARYOTIC CELLS
EP 345,242 December 6, 1989
Assignee: SmithKline Biologicals S. A.

The *gag* gene of human immunodeficiency virus (HIV) was expressed from appropriate promoters in yeast, insect cell culture, and animal cell culture. The gene product forms virus-like particles *in vivo* that are useful for vaccines. Plasmid pRIT13003 containing the coding sequence for the *gag* protein under the control of a polyhedrin promoter was co-transfected into *Spodoptera frugiperda* cells with baculovirus. The recombinant virus containing the *gag* gene was recovered and propagated in *S. frugiperda* cells. Culture supernatants were taken at regular intervals and assayed for the presence of *gag* protein. The protein was detectable with polyclonal antibody as the intact 55 kilodalton protein and as smaller cleavage products. Particles recovered from the medium by centrifugation (100,000 x g, 1 h) also contained the 55 kilodalton protein. The yield of antigen was 80-fold greater in this system than in HTLV-111-infected Molt cells. Similar experiments with the *gag* gene of simian immunodeficiency virus are also reported.

Hansson, L., Josephson, S., and Steiner, H.
A METHOD FOR THE PRODUCTION AND ISOLATION OF A FUSION PROTEIN IN EUKARYOTIC CELLS
EP 356,409 Feb. 28, 1990
Assignee: KabiGen AB

Production and isolation of a fusion protein involves the construction of an expression vector encoding a eukaryotic signal peptide fused to a protein which in turn is fused to a desired protein. The protein fused directly to the signal peptide is capable of selective binding to a carrier material. The expression plasmid may be transformed into compatible eukaryotic cells; after fermentation with the transformants, the fusion protein is isolated by adsorption onto the carrier material. Recombinant baculovirus vectors which encode a tissue plasminogen activator signal sequence-IgG-binding protein-cecropion A fusion protein were prepared. *Spodoptera frugiperda* cells transfected with this vector produced the fusion protein which was purified by IgG-Sepharose affinity chromatography. Cectopin A was released by cleavage with CNBr.

Houston, L.L., Lane, J.A., Piatak, M., and Clark, R.
MANUFACTURE OF RICINS IN RECOMBINANT INSECT CELL CULTURES
WO 8,901,037 February 9, 1989
Assignee: Cetus Corporation

Genes encoding ricin toxin (I), or muteins having reduced galactose binding activity, or subunits thereof, were cloned into a baculovirus transfer vector and co-infected with baculovirus into insect cells where they recombined to produce expression vectors capable of infecting insect cells and expressing the cloned genes. Plasmid pBG46/255, a recombinant baculovirus transfer vector containing a gene encoding ricin B subunit mutein (II) under polyhedrin promoter control, was constructed and co-transfected with wild-type baculovirus DNA into *Spodoptera frugiperda* Sf9 cells. Using this recombinant to infect Sf9 cells resulted in the isolation of 240 ng/ml of II from 5.5 liters of culture medium. This product exhibited little or no binding to galactose.

Kang, C.Y.
EXPRESSION OF HEPATITIS B VIRAL ANTIGENS FROM RECOMBINANT BACULOVIRUS VECTORS
EP 260,090 March 16, 1988

Recombinant baculoviruses containing a gene for hepatitis B surface antigen (HBsAg) or for the precursor (Pre-S2) were prepared. Insect cells infected with these viruses produced 22 nm hepatitis B antigen-containing particles. The gene encoding HBsAg or Pre-S2 was inserted into a plasmid downstream of the polyhedrin promoter to prepare pAcRP6-HBsYK14. Co-transfection of *Spodoptera frugiperda* cells with *Autographa californica* nuclear polyhedrosis virus and this vector resulted in production of recombinant virus AcNPV-HBsYK14. Insect cells infected with this virus produced 22 nm particles indistinguishable by RIA and microscope examination from 22 nm hepatitis B viral antigen.

Kang, C. Y.
IMPROVED BACULOVIRUS EXPRESSION SYSTEM CAPABLE FOR PRODUCTION OF FOREIGN PROTEINS AT HIGH LEVELS
GB 2,228,486, Aug. 29, 1990
Assignee: University of Ottawa

Expression of foreign genes, especially those of human immunodeficiency virus (HIV), in a baculovirus expression system was improved. The heterologous coding sequence was placed immediately adjacent to the 3' end of a putative ribosome binding site and expressed from a polyhedrin promoter. The introduction of the putative ribosome binding site and deletion of the noncoding region of the foreign gene was achieved by a modified crossover linker mutagenesis method. Preparation of AcNPV-HIVKYrev recombinant baculovirus for enhanced expression of the *rev* gene of HIV-1 in the *Spodoptera frugiperda* insect cell line was demonstrated. The rev protein synthesized by the AcNPV-HIVYKrev recombinant virus-infected cells accounted for approximately 20% of the total protein synthesis.

Kawasaki, E.S., Devlin, J.J., Martin, G., O'Rourke, E., and Clark, R.
RECOMBINANT MANUFACTURE OF BIOLOGICALLY ACTIVE COLONY-STIMULATING FACTORS WITH CELL CULTURES OF THE INSECT SPODOPTERA FRUGIPERDA
WO 8,901,038 February 9, 1989
Assignee: Cetus Corporation

Biologically active colony-stimulating factors (CSFs) were manufactured by *Spodoptera frugiperda* cell cultures using baculovirus expression vectors with the cDNA expressed from the baculovirus polyhedrin promoter. CSF1 cDNA was cloned from human pancreatic carcinoma cell line MIA PaCa-2. Plasmid pAcM1 containing this CSF-1 coding sequence under control of the baculovirus polyhedrin promoter was constructed and transfected into cultured cells of *S. frugiperda*. The *S. frugiperda* transformants produced 7500 to 500,000 units CSF/ml and the products were secreted.

Matsuura, Y., Yasui, K., and Sato, T.
JAPANESE ENCEPHALITIS VIRUS ENVELOPE PROTEIN E MANUFACTURE IN RECOMBINANT INSECT TISSUE CULTURE CELLS FOR VACCINES AND DIAGNOSTICS
GB 2,218,421 November 15, 1989
Assignee: Nippon Zeon Co., Ltd.; Tokyo Metropolitan Institute for Neurosciences

A recombinant baculovirus vector was used to express the gene for Japanese encephalitis virus envelope protein E for use in vaccines or diagnostics in insect cell culture. A cDNA encoding the E protein of Japanese encephalitis virus was cloned and introduced into the recombinant vector pAcYMS1 containing the *Autographa californica* polyhedrin gene promoter cloned in pUC8. *Spodoptera frugiperda* Sf9 cells transformed with the recombinant expression vector pAcYMJ3, that carried the J3 fragment encoding the E protein (also preM and M protein) with the polyhedrin promoter upstream, produced E protein in the culture medium. The antigenicity of the recombinant protein E in C3H/He mice was also demonstrated.

Miller, D.W.
PRODUCTION OF HETEROLOGOUS PROTEIN IN INSECTS BY PROPAGATION OF POLYHEDRAL INCLUSION BODIES CONTAINING WILD-TYPE AND RECOMBINANT BACULOVIRUSES
WO 8,802,030 March 24, 1988
Assignee: Genetics Institute, Inc.

When mixed composition polyhedral inclusion bodies (mPIB) was ingested by an insect host, additional copies of the mPIB as well as the desired heterologous protein, were produced. *Spodoptera frugiperda* cells were co-infected with the wild-type polyhedrin-producing *Autographa californica* nuclear polyhedrosis virus (AcNPV) L-1 variant and the recombinant AcNPV 3h8 in which the majority of the polyhedrin structural gene has been deleted and replaced with a human tissue plasminogen activator cDNA. The mPIB's produced were harvested and fed to *Heliothis virenscens* caterpillars. Within 5 days, the caterpillars were homogenized and the progeny PIB's harvested. The progeny PIB's contained both the L-1 and the 3h8 viruses. The ratio of the L-1 to the 3h8 virus increased relative to the original input ratio.

Miller, L.K.
POLYPEPTIDES IN INSECT CELLS
EP 155,476 September 25, 1985
Assignee: Idaho Research Foundation, Inc.

An exogenous gene which codes for a desired protein product was inserted into the chromosome of an insect virus so as to be under the control of a strong gene promoter. The resulting recombinant virus was introduced into insect cells to express the desired protein product. The procedure followed was: (1) the polyhedrin gene region of a nuclear polyhedrosis virus chromosome was cleaved, (2) a passenger gene was inserted into the polyhedrin gene or used to replace it, (3) the resulting plasmid was co-transfected with wild-type viral DNA into suitable host cells, and (4) the host cells were used to express the exogenous gene product. For example, plasmid pEXS942, containing a portion of the *Autographa californica* nuclear polyhedrosis virus DNA, was ligated to plasmid pMC874 containing the β-galactosidase gene of *Escherichia coli* and used for transformation of *E. coli*. The fused plasmid from selected colonies was transferred to the viral genome by transplacement. *Spodoptera frugiperda* cells infected with the mutant virus expressed a polyhedrin/β-galactosidase fusion protein in high yield.

Robson, K.J.H. and Hall, J.R.S.
CLONING AND EXPRESSION OF TRAP GENE OF PLASMODIUM FALCIPARUM MEROZOITE
WO 9,001,496 Feb. 22, 1990
Assignee: 3i Research Exploitation Ltd.

The gene for thrombospondin-related anonymous protein (TRAP) of *Plasmodium falciparum* merozoites was cloned, sequenced, and expressed in insect cell culture. TRAP proteins and fragments thereof may be used as vaccines against malaria. The TRAP gene was identified using a 27-base oligonucleotide based on amino acid sequence PCSVTCGNG in region II of the CS protein. This gene was modified using the polymerase chain reaction and inserted into transfer vector pAcYM1. TRAP expression vector vKKJ17 was prepared by co-transfecting *Spodoptera frugiperda* cells with *Autographa californica* nuclear polyhedrosis virus and pAcYM1. *S. frugiperda* cells infected with this recombinant virus produced TRAP which was purified by ion exchange chromatography and gel filtration.

Roy, P.
PRODUCTION OF BLUETONGUE VIRUS NON-STRUCTURAL PROTEINS USING A BACULOVIRUS EXPRESSION VECTOR
WO 9,002,186 March 8, 1990

Antigenic bluetongue virus (BTV) nonstructural protein (NS) was produced by insect cells infected with recombinant baculovirus. The recombinant NS may be used in vaccine manufacture or in diagnosis of BTV infection in mammals. Recombinant *Autographa californica* nuclear polyhedrosis virus AcBTV10-6 containing cDNA for the 64 kilodalton NS1 protein of the BTV double-stranded RNA middle size segment 6 was prepared. *Spodoptera frugiperda* cells infected with this virus produced NS1 to the extent of approximately 50% of the total stainable extracted protein. The recombinant NS1 protein formed numerous tubular structures in the cytoplasm of the infected cells. The protein was used to identify antibodies to five United States BTV serotypes in infected sheep sera.

Rusche, J., Lynn, D., Carson, H., Putney, S., and Jellis, C.L.
RECOMBINANT HUMAN IMMUNODEFICIENCY VIRUS (HIV) PROTEINS PRODUCED IN INSECT CELLS
EP 272,858 June 29, 1988
Assignee: Repligen Corporation

Recombinant baculoviruses encoding HIV env proteins were prepared. Insect cells containing these recombinant viruses produced gp160env or gp120env. Plasmids encoding gp160env strain RF, a hybrid gp160env protein from strains RF and BH10, and a gp120env from the BH10 strain were constructed. Recombinant baculovirus containing the DNA encoding these proteins were prepared by transfecting *Spodoptera frugiperda* cells with *Autographa californica* nuclear polyhedrosis virus and the plasmids. *S. frugiperda* cells transfected with these recombinant viruses produced gp120env or intact gp160env, i.e., they were not proteolytically processed to gp41 or gp120 and gp41, respectively. Goats immunized with the recombinant gp160env displayed both humoral and cellular immunity to purified gp160env. The neutralizing titers elicited by the recombinant protein was several-fold higher than titers from human AIDS and ARC patients.

Smith, G.E. and Summers, M.D.
A RECOMBINANT BACULOVIRUS EXPRESSION SYSTEM
EP 127,839 May 22, 1984
Related US Patents: US 4,745,051 May 17, 1988; US 4,879,236 November 7, 1989
Assignee: Texas A and M University System

A foreign gene inserted into a plasmid under the transcriptional control of a baculovirus gene promoter was expressed by infected insect cells. The procedure involved: (1) the polyhedrin gene with its promoter from *Autographa californica* nuclear polyhedrosis virus (AcMNPV) was cloned on plasmid pUC8, (2) a *Bam*HI site was constructed within the gene, and (3) human interferon-β-specifying DNA of plasmid pBR13 was inserted at the *Bam*HI site to give the recombinant plasmid pAc380IFN-β. Plasmid pAc380IFN-β was mixed with purified AcMNPV DNA and used to transfect *Spodoptera frugiperda* cells in the presence of Ca^{2+}. Progeny viruses were plaque-purified, and plaques were screened for the occlusion negative phenotype (due to insertional inactivation of the polyhedrin gene). The recombinant viruses recovered were used to infect *S. frugiperda* cells. Infected cells secreted human interferon-β monoclonal antibody-reactive proteins of 17 and 20.5 kdal, corresponding to nonglycosylated and glycosylated human interferon-β.

Smith, R.E., and McGonigal, T.
CLONING, SEQUENCING, AND EXPRESSION OF ROTAVIRUS SA-11 MAJOR CAPSID PROTEIN VP7 cDNA IN A BACULOVIRUS-INSECT CELL EXPRESSION SYSTEM
EP 251,467 January 7, 1988
Assignee: Abbott Laboratories

The recombinant rotavirus SA-11 protein VP7, which may be used to prepare vaccines, was expressed in a baculovirus-insect cell expression system. Rotavirus SA-11 double-stranded RNA was isolated and used to prepare cDNA for the VP7 gene. This cDNA was then used to construct an *E. coli*-baculovirus shuttle vector. The *Spodoptera frugiperda* Sf9 insect cell line was co-transfected with this vector and baculovirus *Autographa californica* to produce the recombinant baculovirus B11.3. B11.3-infected cells secreted a recombinant VP7 protein into the culture medium which could be recognized by anti-rotavirus SA-11 antibodies.

LITERATURE

This section includes the articles referenced in the review portion of this article in addition to a literature survey in the area of the use of the baculovirus expression system for producing recombinant proteins from January 1988 to May 1991. Luckow (*68*) can be consulted for additional articles pertaining to the use of the baculovirus expression system. The major search headings are the same as listed in the patent search. This section is not intended to be all encompassing and lists both review articles and research publications that highlight the varied nature of research in this field during the specified time period.

1. Agathos, S.N., Jeong, Y.-H., and Venkat, K. (1990), Growth kinetics of free and immobilized insect cell cultures. *Ann. NY Acad. Sci.* **589**, 372-398.

2. Atkinson, A.E., Earley, F.G.P., Beadle, D.J., and King, L.A. (1990), Expression and characterization of the chick nicotinic acetylcholine receptor α-subunit in insect cells using a baculovirus vector. *Eur. J. Biochem.* **192**, 451-458.

3. Atkinson, A.E., Weitzman, M.D., Obosi, L., Beadle, D.J., and King, L.A. (1990), Baculoviruses as vectors for foreign gene expression in insect cells. *Pestic. Sci.* **28**, 215-224.

4. Bavarian, F., Fan, L.S., and Chalmers, J.J. (1991), Microscopic visualization of insect cell-bubble interactions. I: rising bubbles, air-medium interface, and the foam layer. *Biotechnol. Prog.* **7**, 140-150.

5. Bergh, M.L.E., Naranjo, C., Mentzer, A.F., Barsomian, G.D., Christopher, C.W., Bartlett, S.H., and Rasmussen, J.R. (1990), in *Glycobiology*, Welply, J.K. and E. Jaworski, E., eds., John Wiley & Sons, Inc., New York, pp. 159-172.

6. Berndt, N. and Cohen, P.T.W. (1990), Renaturation of protein phosphatase 1 expressed at high levels in insect cells using a baculovirus vector. *Eur. J. Biochem.* **190**, 291-297.

7. Bilimoria, S.L. and Carpenter, W.M. (1983), TN-368A: an attached strain of Hink's *Trichoplusia ni* (TN-368) cell line. *In Vitro* **19**, 870-874.

8. Bishop, D.H.L. (1990), Gene expression using insect cells and viruses. *Curr. Opin. Biotechnol.* **1**, 62-67.

9. Blitz, I.L. and Laimins, L.A. (1991), The 68-kilodalton E1 protein of bovine papillomavirus is a DNA binding phosphoprotein which associates with teh E2 transcriptional activator in vitro. *J. Virol.* **65**, 649-656.

10. Brown, M. and Faulkner, P. (1975), Factors affecting the yield of virus in a cloned cell line of *Trichoplusia ni* infected with a nuclear polyhedrosis virus. *J. Invert. Path.* **26**, 251-257.

11. Burns, D.J., Bloomenthal, J., Lee, M.H., and Bell, R.M. (1990), Expression of the α, βII, and γ protein kinase C isozymes in the baculovirus-insect cell expression system. Purification and characterization of the individual isoforms. *J. Biol. Chem.* **265**, 12044-12051.

12. Butters, T.D. and Hughes, R.C. (1981a), Isolation and characterization of mosquito cell membrane glycoproteins. *Biochim. et Biophy. Acta* **640**, 655-671.

13. Butters, T.D., Hughes, R.C., and Vischer, P (1981b), Steps in the biosynthesis of mosquito cell membrane glycoproteins and the effects of tunicamycin. *Biochim. et Biophy. Acta* **640**, 672-686.

14. Cameron, I.R. and Possee, R.D. (1989), Conservation of polyhedrin gene promoter function between *Autographa californica* and *Mamestra brassicae* nulcear polyhedrosis viruses. *Virus Res.* **12**, 183-199.

15. Capone, J.P. and Werstuck, G. (1990), Synthesis of the herpes simplex virus type 1 trans-activator Vmw65 in insect cells using a baculovirus vector. *Mol. Cell. Biochem.* **94**, 45-52.

16. Caron, A.W., Archambault, J., and Massie, B. (1990), High-level recombinant protein production in bioreactors using the baculovirus-insect cell expression system. *Biotechnol. Bioeng.* **36**, 1133-1140.

17. Chalmers, J.J. and Bavarian, F. (1991), Microscopic visualization of insect cell-bubble interactions. II: bubble film and bubble rupture. *Biotechnol. Prog.* **7**, 151-158.

18. Charlton, C.A. and Volkman, L.E. (1991), Sequential rearrangement and nuclear polymerization of actin in baculovirus-infected *Spodoptera frugiperda* cells. *J. Virol.* **65**, 1219-1227.

19. Chen, Y.L. and Israelachvili, J. (1991), New mechanism of cavitation damage. *Science* **252**, 1157-1160.

20. Cho, T., Shuler, M.L., Granados, R.R. (1989), Current developments in new media and cell culture systems for the large-scale production of insect cells. *Adv. Cell Culture* **7**, 261-277.

21. Corsaro, B.G. and Fraser, M.J. (1987), Characterization of clonal populations of the *Heliothis zea* cell line IPLB-HZ 1075. *In Vitro Cell. Develop. Biol.* **23**, 855-862.

22. Crawford, A.M. (1989), Engineering of an Orcytes baculovirus recombinant: insertion of the polyhedrin gene from the *Autographa californica* nuclear polyhedrosis virus. *J. Gen. Virol.* **70**, 1017-1024.

23. Culp, J.S., Johansen, H., Hellmig, B., Beck, J., Matthews, T.J., Delers, A., and Rosenberg, M. (1991), Regulated expression allows high level production and secretion of HIV-1 gp120 envelope glycoprotein in *Drosophila* Schneider cells. *Bio/Technology* **9**, 173-177.

24. Daugherty, B.L., Zavodny, S.M., Lenny, A.B., Jacobson, M.A., Ellis, R.W., Law, S.W., and Mark, G.E. (1990), The uses of computer-aided signal peptide selection and polymerase chain reaction in gene construction and expression of secreted proteins. *DNA Cell Biol.* **9**, 453-459.

25. Davidson, D.J. and Castellino, F.J. (1991a), Asparagine-linked oligosaccharide processing in lepidopteran insect cells. Temporal dependence of the nature of oligosaccharides assembled on asparagine-289 of recombinant human plasminogen produced in baculovirus vector infected *Spodoptera frugiperda* (IPLB-SF-21AE) cells. *Biochem.* **30**, 6167-6174.

26. Davidson, D.J. and Castellino, F.J. (1991b), Structures of the asparagine-289-linked oligosaccharides assembled on recombinant human plasminogen expressed in a *Mamestra brassicae* cell line (IZD-MBO503). *Biochem.* **30**, 6689-6696.

27. 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. *Biochem.* **29**, 5584-5590.

28. de Gooijer, C.D., van Lier, F.L.J., van den End, E.J., Vlak, J.M., and Tramper, J. (1989), A model for baculovirus production and continuous insect cell cultures. *Appl. Microb. Biotechnol.* **30**, 497-501.

29. Doulah, M.S. (1977), Mechanism of disintegration of biological cells in ultrasonic cavitation. *Biotechnol. Bioeng.* **19**, 649-660.

30. Durbin, J.E. and Fallon, A.M. (1985), Transient expression of the chloramphenicol acetyltransferase gene in cultured mosquito cells. *Gene* **36**, 173-178.

31. Eberhard, U. and Schugerl, K. (1987), Investigations of reactors for insect cell culture. *Develop. Biol. Standard.* **66**, 325-330.

32. Fallon, A.M. (1986), Factors affecting polybrene-mediated transfection of cultured *Aedes albopictus* (mosquito) cells. *Exp. Cell Res.* **166**, 535-542.

33. Fallon, A.M. and Willis, J.H. (1985), Gene transfer in insect cells. *Trends Biotechnol.* **3**, 217-218.

34. Felleisen, R., Beck, E., Usmany, J., Vlak, J., Klinkert, M.Q. (1990), Cloning and expression of Schistosoma mansoni protein Sm32 in a baculovirus vector. *Mol. Biochem. Parasitol.* **43**, 289-292.

35. Fiebich, B., Hug, H., and Marme, D. (1990), High-efficiency expression of rat protein kinase C-γ in baculovirus-infected insect cells. *FEBS Lett.* **277**, 15-18.

36. French, T.J., Marshall, J.J.A., and Roy, P. (1990), Assembly of double-shelled, viruslike particles of bluetongue virus by the simultaneous expression of four structural proteins. *J. Virol.* **64**, 5695-5700.

37. Gardner, A.R., Gainer, J.L., and Kirwan, D.J. (1990), Effects of stirring and sparging on cultured hybridoma cells. *Biotechnol. Bioeng.* **35**, 940-947.

38. Gillespie, L.S., Hillesland, K.K., and Knauer, D.J. (1991), Expression of biologically active antithrombin III by recombinant baculovirus in *Spodoptera frugiperda* cells. *J. Biol. Chem.* **266**, 3995-4001.

39. Goldblum, S., Bae, Y.-K., Hink, W.F., and Chalmers, J. (1990), Protective effect of methylcellulose and other polymers on insect cells subjected to laminar shear stress. *Biotechnol. Prog.* **6**, 383-390.

40. Goochee, C.F. and Monica, T. (1990), Environmental effects on protein glycosylation. *Bio/Technology* **8**, 421-427.

41. Goosen, M.F.A., (1991), Large-scale insect cell culture: methods, applications, and products. *Curr. Opin. Biotechnol.* **2**, 365-369.

42. Graf, H. and Schugerl, K. (1991), Influence of the reciprocating movement on the performance of a membrane aeration system in insect cell cultures. *Biotechnol. Techniques* **5**, 91-94.

43. Griffin, W.C. (1949), Classification of surface active agents by HLB. *J. Soc. Cosmet. Chem.* **1**, 311-326.

44. Groebe, D.R., Chung, A.E., and Ho, C. (1990), Cationic lipid-mediated co-transfection of insect cells. *Nucleic Acids Res.* **18**, 4033.

45. Handa, A., Emery, A.N., and Spier, R.E. (1987), On the evaluation of gas-liquid interfacial effects on hybridoma viability in bubble column bioreactors. *Develop. Biol. Standard.* **66**, 241-252.

46. Handa-Corrigan, A., Emery, A.N., and Spier, R.E. (1989), Effect of gas-liquid interfaces on the growth of suspended mammalian cells: mechanisms of cell damage by bubbles. *Enzyme Microb. Technol.* **11**, 230-235.

47. Hasnain, S.E. and Nakhai, B. (1990), Expression of the gene encoding firefly luciferase in insect cells using a baculovirus vector. *Gene* **91**, 135-138.

48. Hink, W.F. (1982), Production of *Autographa californica* nuclear polyhedrosis virus in cells from large-scale suspension cultures. In *Microbial and Viral Pesticides*, E. Kurstak, E., ed., Marcel Dekker, Inc., New York, pp. 493-506.

49. Hink, W.F. and Strauss, E.M. (1980), Semi-continuous culture of the TN-368 cell line in fermenters with virus production in harvested cells. In *Invertebrate Systems In Vitro*, Kurstak, E., Maramorosch, K., and Dubendorfer, A., eds., Elsevier/North Holland Biomedical Press, Amsterdam, pp. 27-33.

50. Hink, W.F., Thomsen, D.R., Davidson, D.J., Meyer, A.L., and Castellino, F.J. (1991), Expression of three recombinant proteins using baculovirus vectors in 23 insect cell lines. *Biotechnol. Prog.* **7**, 9-14.

51. Hoess, A., Moarefi, I., Scheidtmann, H., Cisek, L.J., Corden, J.L., Dornreiter, I., Arthur, A.K., and Fanning, E. (1990), Altered phosphorylation pattern of simian virus 40 T antigen expressed by insect cells by using a baculovirus vector. *J. Virol.* **64**, 4799-4807.

52. Hsieh, P. and Robbins, P.W. (1984), Regulation of asparagine-linked oligosaccharide processing. *J. Biol. Chem.* **259**, 2375-2382.

53. Jarvis, D.L., Fleming, J.G.W., 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.

54. Jarvis, D.L. and Summers, M.D. (1989), Glycosylation and secretion of human tissue plasminogen activator in recombinant baculovirus-infected insect cells. *Mole. Cell. Biol.* **9**, 214-223.

55. Jobses, I., Martens, D., and Tramper, J. (1991), Lethal events during gas sparging in animal cell culture. *Biotechnol. Bioeng.* **37**, 484-490.

56. Kilburn, D.G. and Webb, F.C. (1968), The cultivation of animal cells at controlled dissolved oxygen partial pressure. *Biotechnol. Bioeng.* **10**, 801-814.

57. King, G.A., Daugulis, A.J., Faulkner, P., Bayly, D., and Goosen, M.F.A. (1988), Growth of baculovirus-infected insect cells in microcapsules to a high cell and virus density. *Biotechnol. Lett.* **10**, 683-688.

58. King, G.A., Daugulis, A.J., Goosen, M.F.A., Faulkner, P., and Bayly, D. (1989), Alginate concentration: a key factor in the growth of temperature-sensitive baculovirus-infected insect cells in microcapsules. *Biotechnol. Bioeng.* **34**, 1085-1091.

59. Klaiber, K., Williams, N., Roberts, T.M., Papazian, D.M., Jan, L.Y., and Miller, C. (1990), Functional expression of Shaker potassium channels in a baculovirus-infected insect cell line. *Neuron* **5**, 221-226.

60. Koike, M. and Sato, K. (1988), Culture of insect cell lines originated from *Mamestra brassicase* with autoclaved serum-free medium. In *Invertebrate and Fish Tissue Culture*, Kuroda, Y., Kurstak, E., and Maramorosch, K., eds., Springer-Verlag, New York, pp. 7-10.

61. Kunas, K.T. and Papoutsakis, E.T. (1990), Damage mechanisms of suspended animal cells in agitated bioreactors with and without bubble entrainment. *Biotechnol. Bioeng.* **36**, 476-483.

62. Kuroda, K., Geyer, H., Geyer, R., Doerfler, W., and Klenk, H.-D. (1990), The oligosaccharides of influenza virus hemagglutinin expressed in insect cells by a baculovirus vector. *Virol.* **174**, 418-429.

63. Lery, X. and Fediere, G. (1990), A new serum-free medium for lepidopteran cell culture. *J. Invert. Path.* **55**, 342-349.

64. Licari, P. and Bailey, J.E. (1991), Factors influencing recombinant protein yields in an insect cell-baculovirus expression system: multiplicity of infection and intracellular protein degradation. *Biotechnol. Bioeng.* **37**, 238-246.

65. Litwack, G. (1990), Efficient, low-cost protein factories: expression of human adenosine deaminase in baculovirus-infected insect larvae. *Biochem. Mol. Biol.* **1**, 415-417.

66. Lowe, P.N., Page, M.J., Bradley, S., Rhodes, S., Sydenham, M., Paterson, H., and Skinner, R.H. (1991), Characterization of recombinant human Kristen-ras(4B) p21 produced at high levels in *Escherichia coli* and insect baculovirus expression systems. *J. Biol. Chem.* **266**, 1672-1678.

67. Lowe, P.N., Sydenham, M., and Page, M.J. (1990), The Ha-ras protein p21 is modified by a derivative of mevalonate and methyl-esterified when expressed in the insect/baculovirus system. *Oncogene* **5**, 1045-1048.

68. Luckow, V.A. (1991), in *Recombinant DNA Technology and Applications*, Prokop, A., Bajpai, R.K., and Ho, C.S., eds., McGraw-Hill, Inc., New York, pp. 97-152.

69. Luo, L., Li, Y., and Kang, C.Y. (1990), Expression of gag precursor and secretion of virus-like gag particles of HIV-2 from recombinant baculovirus-infected insect cells. *Virology* **179**, 874-880.

70. Maeda, S., Kawai, T., Obinata, M., Chika, T., Horiuchi, T., Maekawa, K., Nakasuji, K., Saeki, Y., Sato, Y., Yamada, K., and Furusawa, M. (1984), Characteristics of human interferon-α produced by a gene transferred by a baculovirus vector in the silkwork, *Bombyx mori*. *Proc. Japan Acad.* **60(ser. B)**, 423-426.

71. Maeda, S., Kawai, T., Obinata, M., Fujiwara, H., Horiuchi, T., Yoshiyuki, S., Sato, Y., and Furusawa, M. (1985), Production of human α-interferon in silkworm using a baculovirus vector. *Nature* **315**, 592-594.

72. Maiorella, B., Inlow, D., Shauger, A., and Harano, D. (1988), Large-scale insect cell culture for recombinant protein production. *Bio/Technology* **6**, 1406-1410.

73. Martens, J.W.M., Honee, G., Zuidema, D., Van Lent, J.W.M., Visser, B., and Vlak, J.M. (1990), Insecticidal activity of a bacterial crystal protein expressed by a recombinant baculovirus in insect cells. *Appl. Environ. Microbiol.* **56**, 2764-2770.

74. Matsuura, Y., Tatsumi, M., Enami, K., Morikawa, S., and Yamazaki, S. (1989), Expression of IL-6/IFN-β_2 in a baculovirus system and its biological function. *Ann. N.Y. Acad. Sci.* **557**, 122-129.

75. Michaels, J.D., Peterson, J.F., McIntire, L.V., and Papoutsakis, E.T. (1991), Protection mechanisms of freely suspended animal cells (CRL 8018) from fluid-mechanical injury. Viscometric and bioreactor studies using serum, Pluronic F-68, and polyethylene glycol. *Biotechnol. Bioeng.* **38**, 169-180.

76. Miltenburger, H.G. and David, P. (1980), Mass production of insect cells in suspension. *Develop. Biol. Standard.* **46**, 183-186.

77. Mitsuhashi, J. (1989), Nutritional requirements fo insect cells *in vitro*. In *Invertebrate Cell System Applications*, vol. 1, Mitsuhashi, J., ed., CRC Press, Inc., Boca Raton, Florida, pp. 3-20.

78. Mitsuhashi, J. and Goodwin, R.H.(1989), The serum-free culture of insect cells *in vitro*. In *Invertebrate Cell System Applications*, vol. 1, Mitsuhashi, J., ed., CRC Press, Inc., Boca Raton, Florida, pp. 31-43.

79. Murhammer, D.W. and Goochee, C.F. (1988), Scale-up of insect cell cultures: protective effects of Pluronic F-68. *Bio/Technology* **6**, 1411-1418.

80. Murhammer, D.W. and Goochee, C.F. (1990a), Structural features of nonionic polyglycol polymer molecules responsible for the protective effect in sparged animal cell bioreactors. *Biotechnol. Prog.* **6**, 142-148.

81. Murhammer, D.W. and Goochee, C.F. (1990b), Sparged animal cell bioreactors: mechanism of cell damage and Pluronic F-68 protection. *Biotechnol. Prog.* **6**, 391-397.

82. Nagy, E., Huber, P., Krell, P.J., Derbyshire, J.B. (1991), Synthesis of Newcastle disease virus (NDV)-like envelopes in insect cells infected with a recombinant baculovirus expressing the hemagglutinin-neuraminidase of NDV. *J. Gen. Virol.* **72**, 753-756.

83. Noteborn, M.H.M., De Boer, G.F., Kant, A., Koch, G., Bos, J.L., Zantema, A., and Van der Eb, A.J. (1990), Expression of avian leukemia virus env-gp85 in *Spodoptera frugiperda* cells by use of a baculovirus expression vector. *J. Gen. Virol.* **71**, 2641-2648.

84. Oh, S.K.W., Nienow, A.W., Al-Rubeai, M., and Emery, A.N. (1989), The effects of agitation intensity with and without continuous sparging on the growth and antibody production of hybridoma cells. *J. Biotechnol.* **12**, 45-62.

85. Oosterhuis, N.M.G. and Kossen, N.W.F. (1984), Dissolved oxygen concentration profiles in a production-scale bioreactor. *Biotechnol. Bioeng.* **26**, 546-550.

86. Parker, E.M., Kameyame, K., Higashijima, T., and Ross, E.M. (1991), Reconstitutively active G protein-coupled receptors purified from baculovirus-infected insect cells. *J. Biol. Chem.* **266**, 519-527.

87. Passini, C.A. and Goochee, C.F. (1989), Response of a mouse hybridoma cell line to heat shock, agitation, and sparging. *Biotechnol. Prog.* **5**, 175-188.

88. Paul, J.I., Tavare, J., Denton, R.M., and Steiner, D.F. (1990), Baculovirus-directed expression of the human insulin receptor and an insulin-binding ectodomain. *J. Biol. Chem.* **265**, 13074-13083.

89. Peterson, J.F., McIntire, L.V., and Papoutsakis, E.T. (1988), Shear sensitivity of cultured hybridoma cells (CRL-8018) depends on mode of growth, culture age and metabolite concentration. *J. Biotechnol.* **7**, 229-246.

90. Prehaud, D., Harris, R.D., Fulop, V., Koh, C.L., Wong, J., Flamand, A., and Bishop, D.H.L. (1990), Expression, characterization, and purification of a phosphorylated rabies nucleoprotein synthesized in insect cells by baculovirus vectors. *Virology* **178**, 486-497.

91. Radlett, P.J., Telling, R.C., Stone, C.J., and Whiteside, J.P. (1971), Improvements in the growth of BHK-21 cells in submerged culture. *Appl. Microbiol.* **22**, 534-537.

92. Ragona, G., Edwards, S.A., Mercola, D.A., Adamson, E.D., and Calogero, A. (1991), The transcriptional factor Egr-1 is synthesized by baculovirus-infected insect cells in an active, DNA-binding form. *DNA Cell Biol.* **10**, 61-66.

93. Ramakrishna, N., Saikumar, P., Potempska, A., Wisniewski, J.M., and Miller, D.L. (1991), Expression of human Alzheimer amyloid precursor protein in insect cells. *Biochem. Biophys. Res. Commun.* **174**, 983-989.

94. Ramirez, O.T. and Mutharasan, R. (1990), The role of the plasma membrane fluidity on the shear sensitivity of hybridomas grown under hydrodynamic stress. *Biotechnol. Bioeng.* **36**, 911-920.

95. Rasmussen, L., Battles, J.K., Ennis, W.H., Nagashime, K., and Gonda, M.A. (1990), Characterization of virus-like particles produced by a recombinant baculovirus containing the gag gene of the bovine immunodeficiency-like virus. *Virology* **178**, 435-451.

96. Reuveny, S., Velez, D., Macmillan, J.D., and Miller, L. (1986), Comparison of cell propagation methods for their effect on monoclonal antibody production in stirred reactors. *J. Immunol. Methods* **86**, 53-59.

97. Rodewald, H.R., Langhorne, J., Eichmann, K., and Klaus, K.J. (1990), Production of murine interleukin-4 and interleukin-5 by recombinant baculovirus. *J. Immunol. Methods* **132**, 221-226.

98. Roosien, J., Belsham, G.J., Ryan, M.D., King, A.M.Q., and Vlak, J.M. (1990), Synthesis of foot-and-mouth disease virus capsid proteins in insect cells using baculovirus expression vectors. *J. Gen. Virol.* **71**, 1703-1711.

99. Rose, R.C., Bonnez, W., Strike, D.G., and Reichman, R.C. (1990), Expression of the full-length products of the human papillomavirus type 6b (HPV-6b) and HPV-11 L2 open reading frames by recombinant baculovirus, and antigenic comparisons with HPV-11 whole virus particles. *J. Gen. Virol.* **71**, 2725-2729.

100. Shuler, M.L., Cho, T., Wickham, T., Ogonah, O., Kool, M., Hammer, D.A., Granados, R.R., and Wood, H.A. (1990), Bioreactor development for production of viral pesticides or heterologous proteins in insect cell cultures. *Ann. NY Acad. Sci.* **589**, 399-422.

101. Smith, C.G., Greenfield, P.F., and Randerson, D.H. (1989), Effect of micromixing and gas incorporation on suspension culture of hybridomas. In *Proceedings of the Eighth Australian Biotechnology Conference*, University of South Wales, pp. 403-406.

102. Smith, C.M., Hebbel, R.P., Tukey, D.P., Clawson, C.C., White, J.G., Vercellotti, G.M. (1987), Pluronic F-68 reduces the endothelial adherence and improves the rheology of liganded sickle erythrocytes. *Blood* **69**, 1631-1636.

103. Smith, G.E., Summers, M.D., and Fraser, M.J. (1983), Production of human beta interferon in insect cells infected with a baculovirus expression vector. *Mole. Cell. Biol.* **3**, 2156-2165.

104. Svoboda, M., Przybylski, M., Schreurs, J., Miyajima, A., Hogeland, K., and Deinzer, M.(1991), Mass spectrometric determination of glycosylation sites and oligosaccharide composition of insect-expressed mouse interleukin-3. *J. Chromatogr.* **562**, 403-419.

105. Takehara, K., Morikawa, S., and Bishop, D.H.L. (1990), Characterization of baculovirus-expressed Rift Valley fever glycoproteins synthesized in insect cells. *Virus Res.* **17**, 173-190.

106. Thomas, C.P., Booth, T.F., and Roy, P. (1990), Synthesis of bluetongue virus-encoded phosphoprotein and formation of inclusion bodies by recombinant baculovirus in insect cells: it binds the single-stranded RNA species. *J. Gen. Virol.* **71**, 2073-2083.

107. Thomsen, D.R., Post, L.E., and Elhammer, A.P. (1990), Structure of o-glycosidically linked oligosaccharides synthesized by the insect cell line Sf9. *J. Cell. Biochem.* **43**, 67-79.

108. Tramper, J., Williams, J.B., and Joustra, D. (1986), Shear sensitivity of insect cells in suspension. *Enzyme Microb. Technol.* **8**, 33-36.

109. Tramper, J., Joustra, D., and Vlak, J.M. (1987), Bioreactor design for growth of shear-sensitive insect cells. *In*: Plant and Animal Cells (C. Webb and F. Mavituna, eds.), Ellis Horwood Ltd., pp. 125-136.

110. Tramper, J., Smit, D., Straatman, J., and Vlak, J.M. (1988), Bubble-column design for growth of fragile insect cells. *Bioproc. Eng.* **3**, 37-41.

111. Tramper, J., van den End, E.J., de Gooijer, C.D., Kompier, R., van Lier, F.L.J., Usmany, M., and Vlak, J.M. (1990), Production of baculovirus in a continuous insect-cell culture: bioreactor design, operation, and modeling. *Ann. NY Acad. Sci.* **589**, 423-430.

112. Van Bokhoven, H., Wellink, J., Usmany, M., Vlak, J.M. Goldbach, R., and Van Kammen, A. (1990), Expression of plant virus genes in animal cells: high-level synthesis of cowpea mosaic virus B-RNA-encoded proteins with baculovirus expression vectors. *J. Gen. Virol.* **71**, 2509-2517.

113. Van Drunen Littel-Van den Hurk, S., Parker, M.D., Fitzpatrick, D.R., Zamb, T.J., Van den Hurk, J.V., Campos, M., Harland, R., and Babiuk, L.A. (1991), Expression of bovine herpesvirus 1 glycoprotein gIV by recombinant baculovirus and analysis of its immunogenic properties. *J. Virol.* **65**, 263-271.

114. van Lier, F.L.J., van den End, E.J., de Gooijer, C.D., Vlak, J.M., and Tramper, J. (1990), Continuous production of baculovirus in a cascade of insect-cell reactors.

115. Vaughn, J.L. (1976), Production of NPV in large-scale cultures. *J. Invert. Path.* **28**, 233-237.

116. Vaughn, J.L. and Weiss, S.A. (1991), Formulation of media for the culture of insect cells. *BioPharm* **4(2)**, 16-19.

117. Vernet, T., Tessier, D.C., Richardson, C., Laliberte, F., Khouri, H.E, Bell, A.W., Storer, A.C., and Thomas, D.Y. (1990), Secretion of functional papain precursor from insect cells. Requirement for N-linked glycosylation of the pro-region. *J. Biol. Chem.* **265**, 16661-16666.

118. Volkman, L.E. and Summers, M.D. (1975), Nuclear polyhedrosis virus detection: relative capabilities of clones developed from *Trichoplusia ni* ovarian cell line TN-368 to serve as indicator cells in a plaque assay. *J. Virol.* **16**, 1630-1637.

119. Volkman, L.E. and Summers, M.D. (1976), Comparative studies with clones derived from a cabbage looper ovarian cell line, TN-368. In *Invertebrate Tissue Culture: Application in Medicine, Biology, and Agriculture*, Kurstak, E. and Maramorosch, K., eds., Academic Press, New York, pp. 289-296.

120. Wathen, M.W., Aeed, P.A., and Elhammer, A.P., Characterization of oligosaccharide structures on a chimeric respiratory syncytial virus protein expressed in insect cell line Sf9. *Biochem.* **30**, 2863-2868.

121. Weiss, S.A., Smith, G.C., Kalter, S.S., Vaughn, J.L., and Dougherty, E. (1981a), Improved method for the production of insect cell cultures in large volume. *In Vitro* **17(6)**, 495-502.

122. Weiss, S.A., Smith, G.C., Kalter, S.S., Vaughn, J.L., and Dougherty, E. (1981b), Improved replication of *Autographa californica* nuclear polyhedrosis virus in roller bottles: characteristics of progeny virus. *Intervirology* **15**, 213-222.

123. Welply, J.K. (1991), Protein glycosylation: function and factors that regulate oligosaccharide structure. In *Animal Cell Bioreactors*, Ho, C.S. and Wang, D.I.C., eds., Buttersworth-Heinemann, Boston, Massachusetts, pp. 59-72.

124. Wickman, T.J., Davis, T., Granados, R.R., Hammer, D.A., Shuler, M.L., and Wood, H.A. (1991), Baculovirus defective interfering particles are responsible for variations in

recombinant protein production as a function of multiplicity of infection. *Biotechnol. Lett.* **13**, 483-488.

125. Wu, J., King, G., Daugulis, A.J., Faulkner, P., Bone, D.H., and Goosen, M.F.A. (1989), Engineering aspects of insect cell suspension culture: a review. *Appl. Microbiol. Biotechnol.* **32**, 249-255.

126. Wu, J., King, G., Daugulis, A.J., Faulkner, P., Bone, D.H., and Goosen, M.F.A. (1990), Adaptation of insect cells to suspension culture. *J. Ferment. Bioeng.* **70**, 90-93.

127. Wudtke, M. and Schugerl, K. (1987), Investigation of the influence of physical environment on the cultivation of animal cells. *In*: Modern Approaches to Animal Cell Technology (R.E. Spier and J.B. Griffiths, eds.), 8th meeting of the European Society for Animal Cell Technology, pp. 297-315.

128. Zuidema, D., Schouten, A., Usmany, M., Maule, A.J., Belsham, G.J., Roosien, J., Klinge-Roode, E.C., Van Lent, J.W.M., and Vlak, J.M. (1990), Expression of cauliflower mosaic virus gene I in insect cells using a novel polyhedrin-based baculovirus expression vector. *J. Gen. Virol.* **71**, 2201-2209.

129. Zu Putlitz, J., Datta, S., Madison, L.D., Macchia, E., and Jameson, J.L. (1991), Human thyroid hormone β_1 receptor produced by recombinant baculovirus-infected insect cells. *Biochem. Biophys. Res. Commun.* **175**, 285-290.

130. Zu Putlitz, J., Kubasek, W.L., Duchene, M., Marget, M., Von Specht, B.U., and Domdey, H. (1990), Antibody production in baculovirus-infected insect cells. *Bio/Technology* **8**, 651-654.