J. Biosci., Vol. 19, Number 5, December 1994, pp 603–614. © Printed in India.

Baculovirus vector-mediated expression of heterologous genes in insect cells

P SRIDHAR, A K AWASTHI, C A AZIM, S BURMA, S HABIB, A JAIN, B MUKHERJEE, A RANJAN and SEYED E HASNAIN*
Eukaryotic Gene Expression Laboratory, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110 067, India

MS received 13 April 1994; revised 2 August 1994

Abstract. The baculovirus expression system employing *Autagrapha californica* nuclear polyhidrosis virus and *Spodoptera frugiperda* insect cells in culture has proved very popular for high level expression of heterologous genes: In this system, transcription of the foreign gene is usually driven by the hyperactive and temporally regulated polyhedrin gene promoter. Replacement of the polyhedrin gene, which encodes a 29-kDa occlusion protein (non-essential for viral replication), with a gene of interest leads to an occlusion negative phenotype which serves as a visual marker to select for recombinant viruses. Simultaneous expression of multiple genes can also be achieved. The heterologous proteins synthesized in this system are antigenically, immunologically and functionally identical in most respects to their native counterparts. This mini-review will aim at summarizing the potentials and utility of the baculovirus expression vector system and will address some important questions relating to the biology of this system.

Keywords. Baculovirus; heterologous gene; *Autagrapha californica; Spodoptera frugiperda;* insect cells.

1. Introduction

1.1 *The baculovirus expression vector system*

The expression of heterologous genes in insect cells infected with recombinant baculoviruses has become a popular and powerful tool for the synthesis and analysis of a wide variety of eukaryotic and prokaryotic proteins (for reviews see Luckow and Summers 1988; Luckow 1991; Jarvis and Summers 1992; O'Reilly *et al* 1992).

The baculovirus expression vector system (BEVS) uses a group of DNA viruses, the baculoviruses, that infect certain insects and insect cell lines cultured *in vitro.* Late in the virus-infection process, the infected cell devotes much of its energy to the synthesis of a viral protein called polyhedrin that comprises over 50% of the total infected cell protein. Polyhedrin is not essential for the infection process, so it is possible to replace the polyhedrin gene with a heterologous gene while retaining the polyhedrin regulatory signals. Infection of insect cells with such a recombinant baculovirus leads to the synthesis of the foreign protein to high levels.

The method for selection and purification of recombinant viruses has evolved considerably over the last few years. Traditionally recombinants were generated by *in vivo* recombination at the polyhedrin (p29) locus. This was inevitable due to

*Corresponding author.

the inability to manipulate *in vitro* the very large viral genome. The presence of a transfer vector, carrying the foreign gene flanked by viral sequences (present at the polyhedrin locus), during viral replication results in two point cross-overs leading to recombinant progeny viruses which have a replacement of the polyhedrin gene with the foreign gene. These recombinants, however appear at a very low frequency (0·1–1%) rendering screening a tedious, if not difficult, proposition. Alternatives to facilitate the identification of recombinant baculovirus include, use of a co-expressed reporter gene, antibody, PCR screening etc. (O'Reilly *et al* 1992) besides the development of yeast (Patel *et al* 1992) or *in vitro* systems (Peakman *et al* 1992). Recently, a very efficient method for producing recombinant baculovirus at extraordinarily high efficiency (>95%) has been reported (Kitts and Possee 1993). In this method recombinant viruses have a selective advantage simply because an essential gene function is deleted from the wild type virus used for co-transfection which is complemented only after recombination with a transfer vector that carries the foreign gene along with the deleted component of the viral gene.

Baculovirus-infected insect cells perform many of the post-translational and proteolytic processing events seen in higher eucaryotes (O'Reilly *et al* 1992). In most cases, the recombinant proteins are targeted to their natural locations within the cell. Proteins containing signal peptides are recognized and properly cleaved before insertion into cellular membranes or secretion from the cell. The efficiency of secretion can be enhanced further by fusing the honeybee melittin signal peptide (Tessier *et al* 1991). Also, hetero- and homo-oligomeric assemblies have been demonstrated for a wide variety of proteins in baculovirus-infected cells. Using a baculovirus quadruple expression vector, four blue tongue virus proteins have been co-expressed and shown to assemble into virus-like particles in insect cells (Belyaev and Roy 1993). All this, along with the high expression levels that can be achieved (see table 1 for the advantages and disadvantages of this system), accounts for the

Table 1. Salient features of the baculovirus system,

Feature	Advantage	Disadvantage
Eukaryotic environment for protein production	Conducive to almost all the post-translational modifications required for biological activity	(i) Precise nature of modifications differ from those in mammalian cells (ii) Efficiency declines very late in infection
Exceptionally high expression	Up to 1 g protein product per 10^9 cells representing over 30% of the total cellular protein	No guidelines to pre-determine expression levels of a gene, not all genes are expressed to high levels
Expression during late phase	Advantageous for the expression of cytotoxic genes	(i) Modification capacity declines (ii) Continuous culture not possible
Expression at 27°C	Thermolabile proteins can be expressed	Cold-sensitive proteins may not be expressed
Capacity for large insertions	Large number of genes can be expressed simultaneously, over 10 kb DNA can be inserted	Large DNA would be fragile *in vitro*, stability of the recombinant viruses is a potential problem
Gene splicing	Baculovirus system can carry out splicing	cDNAs recommended for efficient expression
Simplicity of technology	Helper-virus independent, simple to use	Time requirements are longer

immense popularity of BEVS which is evident from the fact that more than 500 genes from viruses, bacteria, fungi, plants, and animals have been already been expressed in this system.

1.2 *Baculovirus infection process*

Baculoviruses specifically infect lepidopterans and usually have a limited host range which however, could be extended by manipulating the viral helicase gene (Croizier *et al* 1994). *Autographa californica* nuclear polyhedrosis virus (A*c*NPV) is the prototype virus which has been fairly well characterized. The A*c*NPV genome consists of a circular, double stranded, covalently closed DNA of ~130kbp. The viral infection cycle can be divided into three main phases—early, late and very late. Transcription of all these classes of baculovirus genes occurs in a coordinately regulated and highly ordered cascade (Rice and Miller 1986). During the early phase, there is transcription of genes whose products are essential for viral DNA replication. The early genes are transcribed, in the absence of viral gene expression (Crawford and Miller 1988), by the host RNA polymerase II (Grula *et al* 1981). The late phase witnesses the transcription of genes encoding structural proteins and budding of nucleocapsids (Fraser 1986). In the very late phase, there is hyper-expression of the polyhedrin and pl0 genes which are important for the formation of the occlusion matrix of the virions. During the late and very late phases, transcription from the p29/pl0 promoters is mediated by an α-amanitin resistant RNA polymerase which may be a virus-modified host RNA polymerase or a virus encoded polymerase (Huh and Weaver 1990).

2. Regulation of the very late promoters: involvement of host protein factor(s) and upstream sequences

The polyhedrin promoter has an unusual structure and shares sequence homology only with some yeast mitochondrial promoters but like other baculovirus late and very late promoters, it carries a strongly conserved tetra nucleotide motif (TAAG) which is a primary determinant of promoter activity (Rohrmann 1986; Thiem and Miller 1989). This sequence lies within a strongly conserved octanucleotide motif at the transcriptional start point (tsp) unlike other promoters which carry two or more such sequences upstream or downstream to the tsp. The requirement of the 5'-untranslated region for high level expression (Guarino and Summers 1987) is yet another interesting feature of this promoter. The regulation of the polyhedrin promoter by *cis* acting elements and *trans-acting* factors that bind to the promoter is currently an open question. A host factor isolated from the lepidopteran *Spodoptera frugiperda* (Sf21) cell line has been recently found to bind with very high specificity and affinity to transcriptionally important motifs within the polyhedrin promoter (Burma *et al* 1994). This protein, called the polyhedrin promoter-binding protein (PPBP), has a molecular weight of 30 kDa and formed a DNA: protein complex that was stable at salt concentrations up to 2 M NaCl and at temperatures as high as 65°C and had a dissociation constant in the picomolar range. Interestingly, PPBP requires phosphorylation for binding because dephosphorylation eliminates complex formation.

PPBP was also found to be present in Bm5 cells (derived from *Bombyx mori*). This cell line has previously been demonstrated to be non-permissive for A*c*NPV replication and transcription. Surprisingly, PPBP isolated from B5 formed a complex with the polyhedrin promoter, which had an increased electrophoretic mobility compared to the complex formed by PPBP from Sf21 cells (figure 1). The molecular weight of PPBP from both permissive and non-permissive cell lines, however, was identical in a UV cross-linking experiment indicating that additional factors may be involved in the interaction of PPBP with the promoter in the transcription permissive cell lines.

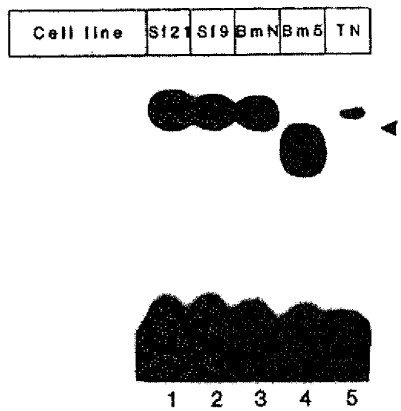

Figure 1. Gel retardation assay using nuclear protein extract from five insect cell lines. Gel retardations were performed using 2 μg of each nuclear protein sample and a radio labelled 32 bp oligonucleotide probe corresponding to the A*c*NPV polyhedrin gene promoter containing the PPBP-binding site (Burma *et al* 1994). Lanes 1-5 show DNA-protein complexes (denoted by an arrowhead) obtained from Sf21, Sf9, BmN, Bm5 and TN368 cells, respectively. The origins of the different cell lines are; *Spodoptera frugiperda* (Sf21, Sf9), *Bombyx mori* (BmN, Bm5) and *Trichoplusia ni* (TN368).

The pl0 promoter, another very late promoter, has also been used for expression of a number of genes. Using similar experimental approaches, PPBP was shown to exhibit specific binding to the p10 promoter. However, there were differences in PPBP binding in the untranslated leader regions of these two very late promoters in heterologous cold competition assays (Jain A, Burma S and Hasnain S E, in preparation). The involvement of a host factor in transcription from the very late polyhedrin gene promoters which in turn requires virus-specific trans-acting factors is presently an enigma. PPBP which has features resembling the eukaryotic TATA-binding protein (TBP) may conceivably play a key role in mediating its effect through protein-protein interaction(s).

Linker scan mutations have demonstrated that the regions important for polyhedrin promoter activity lie within a 92 bp stretch (Ooi *et al* 1989) located immediately upstream to the ATG of the polyhedrin gene. Using a simple, lipofectin based transient expression system with the firefly luciferase gene as a reporter (figure 2), we have observed that sequences within 4 kb upstream region influence transcription from the polyhedrin promoter. BAL31 deletion analyses identified two regions

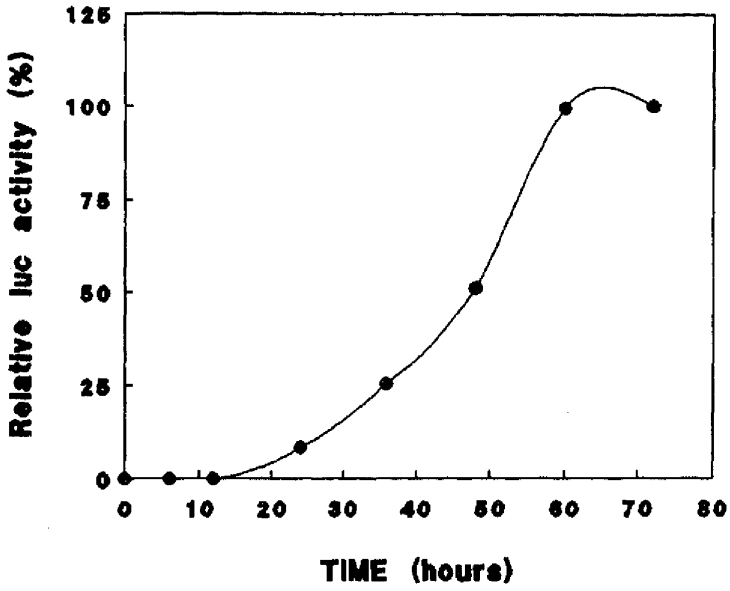

Figure 2. Temporal profile of luciferase expression in a lipofectin based transient expression system. The luciferase reporter gene under the transcriptional control of the polyhedron gene promoter follows the normal very late activation profile of the polyhedrin gene in the presence of wild type *Ac*NPV infection (MOI of 10). Luciferase activity, measured in a luminometer, appears from 18 h post infection and peaks around 60 h post infection. No luciferase activity was registered in the absence of viral infection.

(region I and region II), spanning 0 to 1·1 and 1·5 to 3·12 map units on the *Eco*RI "I" fragment of the viral genome (figure 3). Deletion of these sequences resulted in a 72% and 96% reduction respectively in reporter gene expression (Habib S, Azim C A, Burma S and Hasnain S E, unpublished results). Regulation of the polyhedrin promoter by neighboring *cis*-acting sequence elements is therefore, another facet which has generated a lot of interest. Experiments using subtraction library based expression have likewise identified other sequences from the A*c*NPV genomic library, termed as late expression factors *(lef*-1, *lef*-2, *lef*-3, *lef*-4, *lef*-5, *lef*-6, and *lef*-7) which are important for very late expression (Passarelli and Miller 1993a,b,c; Li *et al* 1993; Morris *et al* 1994). These sequences have been shown to be essential, but not sufficient, for very late expression though their mechanism of action has not been elucidated.

3. Secretion and processing of expressed protein in cell lines and caterpillars

Lepidopteran cells are capable of performing most post-translational modifications (table 1) such as proteolytic processing, N-terminal blocking, glycosylation, phosphorylation, myristylation, oligomerization and assembly resulting in a protein which invariably retains full functional characteristics (Luckow 1993). However, *Ac*NPV infection results in a shut-off of host gene expression by 24 h pi (Carstens *et al* 1979). This may explain the apparent decline in the host ER functions during

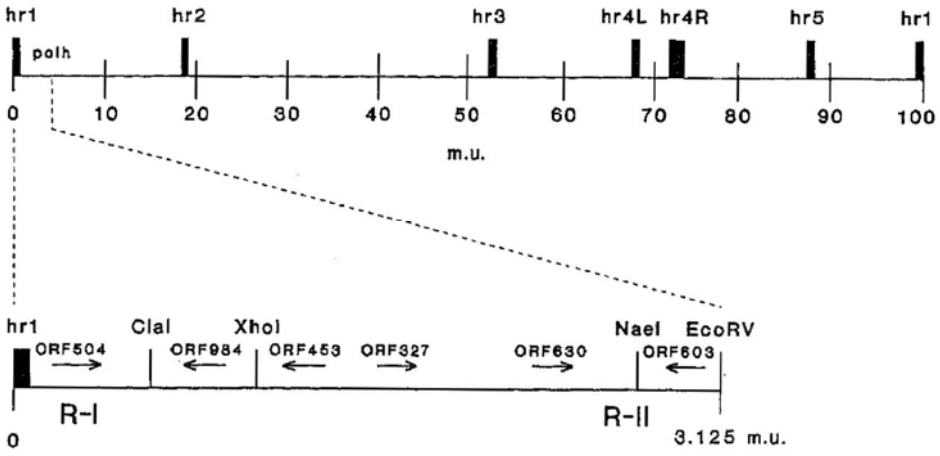

Figure 3. Linear map of the *Ac*NPV genome represented in % map units. The distribution and position of the homologous regions (*hr*) are indicated by vertical filled bars. The lower panel depicts the *Eco*RI-"I" fragment containing sequences upstream to the polyhedron gene promoter. Arrows indicate the location and the orientation of the ORFs in this region. The two regions (R-I and R-1I) identified after BAL31 deletion analyses are indicated.

the very late phase of infection (Jarvis and Summer 1989). Hence, the rate of movement and extent of glycosylation of heterologous gene products synthesized under polyhedrin promoter control appear to decline between 24 and 48 h pi (Jarvis and Summers 1989). This is more evident in the expression of an extensively processed and secretory glycoprotein such as the human chorionic gonadotropin (hCG) hormone, where expression under the control of the polyhedrin promoter may result in partially processed or incompletely secreted product. This phenomenon of "secretory load" (Nakhai *et al* 1991; Nakhai *et al* 1992) faced by the host cells in processing a protein at very late time points of infection when the cellular machinery is severely compromised by the lytic nature of the baculovirus infection has been extensively investigated. Using the expression of the subunits of hCG in both insect cells and larvae it was shown that the hormone which was synthesized to very high levels (~ 11 μg/2 × 10^6 cells/ml) was both immunoreactive and bioactive but was incompletely secreted, with a large percentage of the protein being retained within the cell. Experimental strategies were devised to provide cells enough time after expression to completely process and secrete the protein. The first strategy involved advancing the time of transcription of the gene by using a promoter (core promoter) activated earlier in the viral life cycle than the very late polyhedrin gene promoter. A comparative evaluation of the extent of secretion and quality of the βhCG synthesized under polyhedrin promoter control (βhCG_{P0L}) and that under the core promoter control (βhCG_{COR}) provided interesting results. βhCG_{C0R}, synthesized at earlier time points post infection was secreted to higher levels than βhCG_{P0L} by virtue of increased time available before cell lysis. βhCG_{C0R} was also glycosylated to a higher degree and consequently showed a higher level of bioactivity per unit mass of protein than βhCG_{P0L} (Sridhar *et al* 1993). The affinity purified, recombinant βhCG had a high mannose pattern of glycosylation and was additionally sialylated (Sridhar et al 1993; Sridhar and Hasnain 1993) and once again βhCG_{C0R} was

quantitatively more sialylated than $\beta hCG_{P0L.}$ This relative difference in bioactivity was a direct reflection of the quality and quantity of sugars added post-translationally to the nascent βhCG polypeptides. This differential expression strategy additionally served as a model to investigate the role of sugars *vis-a-vis* protein structure and function.

Another strategy to overcome the secretory load involved the use of the yeast *cdc*2 gene product which has been shown to delay lysis of infected cells. Using a dual recombinant virus carrying the genes for βhCG and *cdd*2 (Sridhar 1993), it was shown that the recombinant βhCG (βhCG_{CDC}) was not only secreted over a longer period of time but was also immunoreactive (figure 4) and more bioactive than $\beta hCG_{POL.}$ This was by virtue of the prolonged period of time available to the infected cells, due to the effect of the *cdc*2 gene product, facilitating the processing of the recombinant protein. The precise mode of action of the *cdc*2 protein—a protein kinase involved in cell cycle—in delaying virus induced cellular lysis is, however, not known.

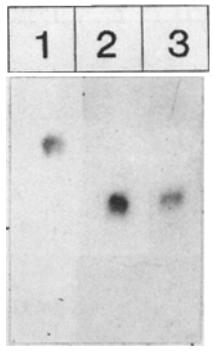

Figure 4. Characterization of the recombinant βhCG by Western blot analysis. Lane 2 represents the cell pellet and lane 3 represents the culture supernatant of v*Accdc*β*hCG*-infected cells which were fractionated on a 12·5% SDS-PAGE and blotted with a βhCG specific monoclonal antibody (Sridhar *et al* 1993). Standard urinary βhCG protein (lane 1) was used as control.

Live caterpillars have been proposed and successfully used as natural host for baculovirus mediated expression of foreign genes. Insect larvae are perhaps more suitable for the baculovirus-mediated synthesis of extensively modified and processed heterologous proteins because the virus encoded *egt* gene causes a delay in molting of the virus-infected larvae (O'Reilly and Miller 1989), thereby prolonging the time available for protein processing. This natural mode of bypassing the secretory load was experimentally exploited in the expression of the βhCG gene. The convenience of assaying the firefly luciferase reporter gene (Hasnain and Nakhai 1990) was used for initial standardization of the larval system (Jha *et al* 1990, 1992a, b) . A dual recombinant virus *vAc* β*hCG-luc,* carrying the genes for βhCG and luciferase under identical transcriptional controls of two polyhedrin promoters placed back to back (Hasnain *et al* 1994), was used to infect *Spodoptera* larva and, upon injection of luciferin on the fourth day after infection, was found to fluorescence (figure 5)

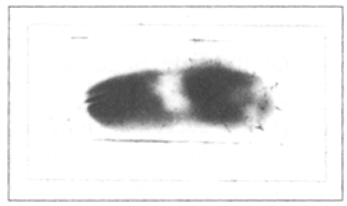

Figure 5. Whole body fluorescence of *Spodoptera* larvae infected with a recombinant baculovirus containing firefly luciferase gene. The fluorescence of the larvae, reared on castor leaves and infected with the recombinant virus as described earlier (Jha *et al* 1990), was captured by injecting luciferin and placing Kodak OG-1 X-ray film underneath the infected caterpillars (72 h post infection).

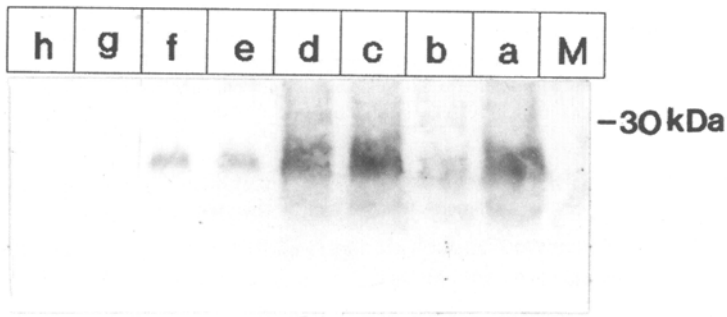

Figure 6. Western blot analysis of larval-derived βhCG, Lanes a and b represent hemolymph and body tissue of *vAc*β*hCG*$_{COR}$ infected larvae. Lanes c and d represent hemolymph and body tissue of *vAc* â*hCG*POL infected larvae. Lanes e and f represent βhCG from culture supernatant and cell pellet of infected cells while lanes g and h represent mock-infected larval hemolymph and homogenate. (M designates the protein molecular mass marker).

due to the synthesis of the luciferase protein in almost all the cells of the larva. βhCG simultaneously synthesized by the same larva was harvested from the hemolymph of infected larvae and was found to be immunoreactive (figure 6). An evaluation of the larval derived βhCG revealed increased secretion of the βhCG$_{C0R}$ in the hemolymph (figure 7). Likewise, direct comparison of the larval βhCG$_{COR}$ and βhCG$_{POL}$ revealed that the former showed higher levels of bioactivity as evident from both receptor binding and testosterone stimulation in a mouse Leydig cell assay (figure 8).

4. Other factors influencing expression of a foreign gene in BEVS

Foreign gene expression in the baculovirus system has been shown to be influenced by the host insect cell line used (Iatrou and Meidinger 1990). Cell line-dependent expression of foreign genes in the baculovirus system is therefore another area of investigation. A dual recombinant *vAc*β*hCG-luc* virus carrying two reporter

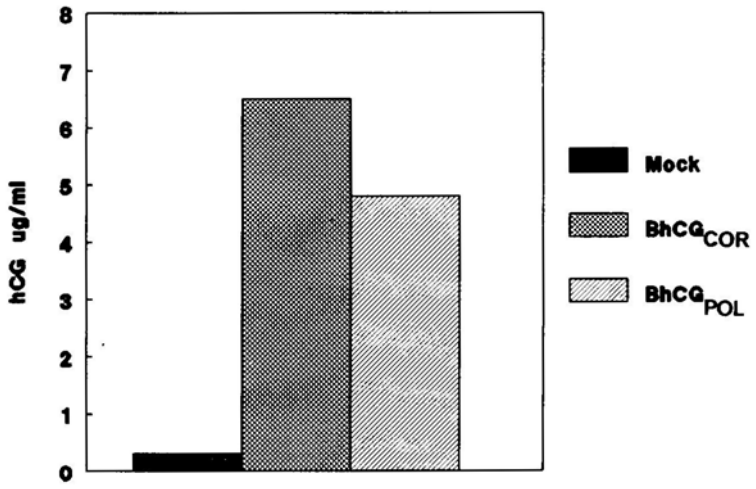

Figure 7. Radioimmunoassay of hemolymph of *Spodoptera* larvae infected with high titres of *vAc*βh*CG*$_{POL}$ or *vAc*β*hCG*$_{COR}$ RIA was carried out as described earlier (Nakhai 1992) using anti-βhCG antibody.

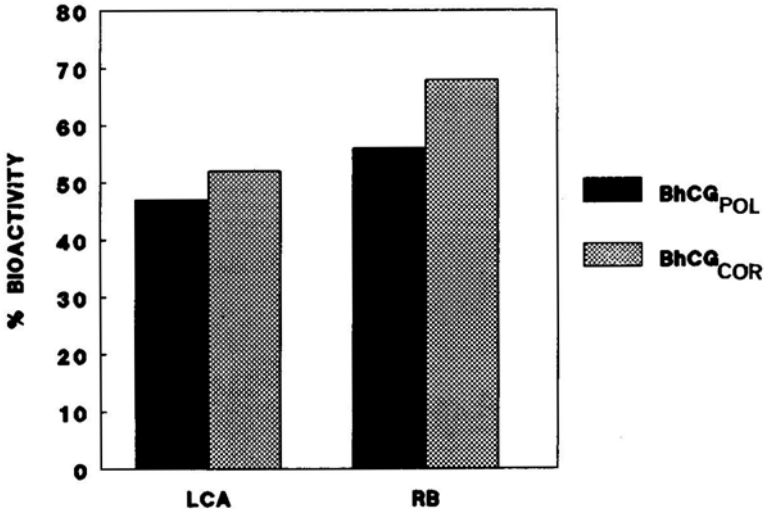

Figure 8. Biological activity of βhCG from *Spodoptera* larvae infected with *vAc*β*hCG*$_{POL}$ and *vAc*β*hCG*$_{COR}$. Hemolymph from infected larvae was withdrawn and estimated for the presence of βhCG using a subunit specific RIA. Equal amounts of βhCG were used in an annealing reaction with a standard preparation of αhCG and the resulting dimer was checked for bioactivity (Nakhai *et al* 1991) by receptor binding (RB) or using a mouse Leydig cell assay (LCA).

genes—βhCG and *luciferase* placed under the transcriptional control of the AcNPV polyhedrin gene promoters was used to infect a series of lepidopteran cell lines derived from *S. frugiperda* (Sf21 and Sf9), *B. mori* (BmN and Bm5) and *Trichoplusia*

ni (TN368). TN368 expressed βhCG to maximum levels (12·5 μg βhCG/ml/2 × 10^6 cells), followed by BmN (9·8 μg), Sf21 (8·7 μg) and Sf9 (7·7 μg) in descending order, with a similar pattern for *luc* (Mukherjee *et al* 1994). Bm5 did not show any evidence of synthesis of the two proteins. In an effort to understand this cell line mediated difference in expression, the status of viral DNA and transcription of the same in these cell lines were analysed. Dot blot analysis of DNA from the *vAcβhCG-luc* infected cells revealed that the level of entry of viral DNA was the same for all the five cell lines and after the completion of viral DNA replication (18 h post infection) the level of viral DNA was also identical, except for Bm5 where viral DNA replication did not take place and the residual virus was cleared from the cells within 6 h of infection. Analysis of RNA from the four expressing cell lines revealed a direct correlation of protein levels with levels of mRNA suggesting transcriptional control.

Using *vAcβhCG-luc,* a dramatic difference was also observed between the expression level of βhCG and that of luciferase though the two genes were under identical transcriptional controls (Hasnain *et al* 1994). The significant differences in the secondary structures of the two genes could possibly explain the apparent differences in their level of expression (Hasnain *et al* 1994). A computer analysis of genes expressed in BEVS and comparison with the viral gene additionally revealed major differences at the level of codon usage and the initiation codon context. Genes which were over expressed had a pattern somewhat similar to that of *Ac*NPV while those which were under expressed exhibited deviations from the pattern seen for *Ac*NPV (Ranjan and Hasnain 1994).

5. Epilogue

It is apparent from the foregoing discussions that although the baculovirus system is being extensively used, the processes that govern high level expression remain to be understood. The viral gene(s) encoding lytic functions have not yet been identified. Our knowledge about the regulation of the very commonly used polyhedrin gene promoter both in terms of the DNA sequence requirements and the involvement of factors, cellular as well as viral is, at best, scanty. Once elucidated, they could go a long way in improving this system and also developing a virus-free expression system. Besides, the insect cellular processing machinery and the molecular events associated with post-translational modifications of heterologous proteins constitute the unknown paradoxes of the baculovirus system.

Acknowledgements

Research in the Eukaryotic Gene Expression laboratory was supported by grants to SEH from the Department of Biotechnology (DBT), New Delhi. PS, SB, SH, AJ, BM and AR thank the Council of Scientific and Industrial Research, New Delhi, for Research Fellowships. AKA was supported by a National Associateship from the DBT. We would like to thank the past members of the EGE lab for their contributions.

References

Belyev A S and Roy P 1993 Development of baculovirus triple and quadruple expression vectors: co-expression of three or four blue tongue virus proteins and the synthesis of blue tongue virus-like particles in insect cells; *Nucleic Acids Res.* **21** 1219–1223

Burma S, Mukherjee B, Jain A, Habib S and Hasnain S E 1994 An unusual 30 kDa protein binding to the polyhedrin gene promoter of the *Autographa californica* nuclear polyhedrosis virus; J. *Biol. Chem.* **269** 2750–2757

Carstens E B, Tjia S T and Doerfler W 1979 Infection of *Spondoptera frugiperda* cells with *Autographa californica* nuclear ploy hedrosis virus. Synthesis of intracellular proteins after virus infection; *Virology* **99** 386–398

Crawford A M and Miller L K 1998 Characterization of an early gene accelerating expression of lategenes of the baculovirus *Autographa californica* nuclear Polyhedrosis virus; *J. Virol.* **62** 2773–2781

Croizier G, Croizier L, Argaud O and Poudevigne D 1994 Extension of *Autographa californica* nuclear polyidrosis virus host range by inter-specific replacement of a short DNA sequence in the p 143 helicase gene; *Proc. Natl. Acad. Sci. USA* **91** 48–52

Fraser M J 1986 Ultra structural observations of virion maturation in *Autographa Californica* nuclear polyhedrosis virus infected *Spodoptera frugiperda* cell cultures; *J. Ultrastruct. Mol. Struct. Res.* **95** 189–195

Grula M A, Buller P I and Weaver RF 1981 α-amanitin resistant viral RNA synthesis in nuclei isolated from nuclear ploy hedrosis virus infected *Hellothis* larval and *Spodoptera frugiperda* cells; *J. Virol.* **38** 916–921

Guarino L A and Summers M D 1987 Nucleotide sequence and temporal expression of a baculovirus regulatory gene; *J. Virol.* **61** 2091–2099

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

Hasnain S E, Nakhai B, Ehtesham N Z, Sridhar P, Ranjan A, Talwar G P and Jha P K 1994 β-subunit of human chorionic gonadotropin hormone and firefly uciferase simultaneously synthesized in insect cells using a recombinant baculovirus are differentially expressed and transported; *DNA Cell Biol.* **13** 275–282

Huh N E and Weaver R F 1990 Identifying the RNA polymerases that synthesize specific transcripts of the *Autographa californica* nuclear polyhedrosis virus; *J. Gen. Virol.* **71** 195–202

Latrou K and Meidinger R G 1990 Tissue specific expression of silk moth chorion genes *in vivo* using *Bombyx mori* nuclear polyhedrosis virus as a transducing vector; *Proc. Natl. Acad. Sci. USA* **87** 3650–3654

Jarvis D L and Summers M D 1989 Glycosylation and secretion of human tissue plasminogen, activator in recombinant baculovirus infected insect cells; *Mol. Cell. Biol.* **9** 214–223

Jarvis D L and Summers M D 1992 Baculovirus expression vectors; in *Recombinant DNA vaccines*; *Rationale and strategy (ed.)* R E Issacson (New York: Marcel Dekker) pp 265–291

Jha P K, Qadri A and Hasnain S E 1992a A monoclonal antibody against firefly luciferase enzyme; *Indian J. Exp. Biol.* **31** 106–108

Jha P K Pal R Nakhai B, Sridhar P and Hasnain S E 1992b Simultaneous synthesis of enzymatically active luciferase and biologically active β-subunit of human chorionic gonadotropin in caterpillars infected with a recombinant baculovirus; *FEBS Lett.* **310** 148–152

Jha P K Nakhai B, Sridhar P, Talwar G P and Hasnain S E 1990 Firefly luciferase synthesized to very high levels in caterpillars infected with a recombinant baculovirus can also be used as an efficient reporter enzyme *in vivo; FEBS Lett.* **274** 23–26

Kitts P A and Possee R D 1993 A method for producing recombinant baculovirus expression vectors at high frequency; *Biotechniques* **14** 810–817

Li Y, Passarelli A L and Miller L K 1993 Identification, sequence and transcriptional mapping of *lef-3,* a baculovirus gene involved in late and very late gene expression; *J. Virol.* **67** 5260–5208

Luckow V A and Summers M D 1988 Trends in the development of baculovirus expression vectors; *Bio/Technology* **6** 47–55

Luckow V A 1991 Cloning and expression of heterologous genes in insect cells with baculovirus vectors; in *Recombinant DNA technology and applications* (eds) A Prokop, R K Bajpai and C Ho, (New York: McGraw-Hill) pp 97–152

Luckow V A 1993 Baculovirus systems for the expression of human gene products; *Curr. Opin.*

Biotechnol. **4** 564–572

Morris T D, Todd J W, Fisher B and Miller L K 1994 Identification of *lef*-7: A baculovirus gene affecting late gene expression; *Virology* **200** 360–369

Mukherjee B, Burma S, Talwar G P and Hasnain S E 1994 Transcriptional regulation of cell line-dependent, baculovirus-mediated expression of foreign genes; *DNA Cell Biol,* (in press)

Nakhai B 1992 *Studies on expression of gonadotropin genes in insect cells using baculovirus expression vector system* Ph.D, thesis, Jawaharlal Nehru University, New Delhi

Nakhai B, Pal R, Sridhar P, Talwar G P Hasnain S E 1991 The alpha-subunit of human chorionic gonadotropin hormone synthesized in insect cells using a baculovirus vector is biologically active; *FEBS Lett.* **283** 104–108

Ooi B G, Rankin C and Miller L K 1989 Downstream sequences augment transcription from essential intiation site of a baculovirus polyhedrin gene; *J. Mol. Biol.* **210** 721–730

O'Reilly D R and Miller L K 1989 A baculovirus blocks insect molting by producing ecdysteroid UDP-glucosyl transferase; *Science* **245** 1110–1112

O'Reilly D R, Miller L K and Luckow V A 1992 *Baculovirus expression vectors—A laboratory manual* (New York: W H Freeman)

Passarelli A L and Miller L K 1993a Identification and characterization of *lef*-1*,* a baculovirus gene involved in late and very late gene expression; *J. Virol.* **67** 3481–3488

Passarelli A L and Miller L K 1993b Three baculovirus genes involved in late and very late gene expression: *ie-1, ie-n,* and *lef-2; J. Virol.* **67** 2149–2158

Passarelli A L and Miller L K 1993c Identification of genes encoding late expression factors located between 56.0 and 65.4 map units of the *Autographa californica* nuclear polyhedrosis virus genome; *Virology* **197** 704–714

Patel G K Nasmyth and N Jenes 1992 A new method for the isolation of recombinant baculovirus; *Nucleic Acids Res.* **20** 97–104

Peakrnan T C, Karris R A and Gewert D R 1992 Highly efficient generation of recombinant baculoviruses by enzymatically mediated site-specific *in vitro* recombination; *Nucleic Acids Res.* **20** 495–500

Ranjan A and Hasnain S E 1994 influence of codon usage and translation initiation codon context in the AcNPV-based expression system: Computer analysis using homologous and heterologous genes; *Virus Genes* (in press)

Rice W C and Miller L K 1986 Baculovirus transcription in the presence of inhibitor and in non-permissive *Drosophila* cells; *Virus Res.* **6** 155–172

Rohrmann G F 1986 Polyhedrin structure; *J. Gen. Virol.* **67** 1499–1514

Sridhar P 1993 *Expression of gonadotropin genes under different promoterrs in insect cells and larvae*; Ph.D, thesis, Jawaharlal Nehru University, New Delhi

Sridhar P, Panda A K, Pal R, Talwar G P and Hasnain S E 1993 Temporal nature of the promoter and not relative strength determines the expression of an extensively processed protein in a baculovirus system; *FEBS Lett.* **315** 282–286

Sridhar P and Hasnain S E 1993 Differential secretion and glycosylation of recombinant human chorionic gonadotropin (βhCG) synthesized using different promoters in the baculovirus expression vector system; *Gene* **131** 261–264

Tessier D C, Thomas D Y, Khouri H E, Laliberte F and Vernet T 1991 Enhanced secretion from insect cells of a foreign protein fused to the honeybee melitin signal peptide; *Gene* **98** 177–183

Thiem S M and Miller L K 1989 Identification, sequence and transcriptional mapping of the major capsid protein gene of the baculovirus *Autographa californica* nuclear polyhedrosis virus; *J. Virol.* **63** 2008–2018