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Review

Expression of genes coding for animal virus glycoproteins in heterologous systems*

Krystyna Bieńkowska-Szewczyk and Bogusław Szewczyk✉

Department of Molecular Virology, Faculty of Biotechnology, University of Gdańsk, Kładki 24, 80-822 Gdańsk, Poland

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The outermost layers of animal viruses are usually composed of glycoproteins. They are responsible not only for the entrance of viruses into, and release from host cells but also for the initial interaction of a viral particle with immunological defense of the host. It is therefore not surprising that many laboratories devote a lot of effort to study viral glycoproteins at the molecular level. Very often such studies are possible only after the introduction of a glycoprotein gene into a heterologous system. Expression of glycoprotein genes is usually obtained in mammalian or insect cells. Expression in mammalian cells yields viral glycoproteins with glycan chains indistinguishable from the original counterparts in virion particles but the level of synthesis of glycoproteins is very low. Vaccinia virus is the most common vector for expression in mammalian cells. It is easy to grow, the introduction of foreign genes is relatively simple and, due to the size of the vaccinia genome, it can accept large pieces of foreign DNA. Glycosylation in insect cells is not as complex as in mammalian cells and usually glycoproteins produced in insect cells are of slightly lower molecular mass than those produced in mammalian cells. The most common vector for expression of glycoproteins in insect cells is a baculovirus, *Autographa californica* nuclear polyhedrosis virus (AcNPV). The great advantage of this system is a very high level of expression of foreign genes.

Recombinant DNA technology has made it possible to produce viral glycoproteins in heterologous systems. The synthesis of a glycoprotein in an isolated system is very often cru-

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✉Corresponding author: tel.: (48 58) 301 5741, fax: (48 58) 301 0072;
e-mail: szewczyk@biotech.univ.gda.pl

Abbreviations: AcNPV, *Autographa californica* nuclear polyhedrosis virus; BV, budded virus particles; EEV, extracellular enveloped virus; IEV, intracellular enveloped virus; OV, occluded viral particles; TK, thymidine kinase.

cial to the understanding of its biological role and to the studies of its physicochemical properties. Taking into account that viral glycoproteins are the components of the outer shell of a virion particle, it is obvious that they are the main target of the host immune response. Therefore, they are potential candidates for subunit vaccines and for antigens in immunodiagnostic tests. Since the advent of recombinant DNA techniques, many laboratories tried to express viral glycoprotein genes for these purposes and for the studies of their role in the penetration of viruses into host cells. The initial attempts to express glycoproteins in bacterial systems were usually unsuccessful. Lack of glycosylation in bacteria resulted in the production of unmodified polypeptide chains which rarely exhibited properties of native glycoproteins. The development of eukaryotic expression systems made it possible to produce glycoproteins similar to, or identical with their natural counterparts. All kinds of eukaryotic expression systems have been used for the expression of glycoproteins depending upon the planned application of the product. A search for the data on the type of systems used for the expression of glycoprotein genes reveals that vaccinia and baculovirus expression sytems are the most popular, although other systems such as yeast, adenovirus or herpesvirus expression systems are also frequently used. These two most popular systems will be discussed in more detail in this minireview.

BIOLOGY OF VACCINIA VIRUS

Vaccinia virus belongs to the family Poxviridae which comprises the largest and most complex animal viruses. The size of double-stranded genome is over 180 kb and it contains over 250 potential genes (Goebel *et al.*, 1990; Moss, 1991). Virions are enveloped and they are of a characteristic brick shape. They are so large that they can be seen in a good quality light-microscope. Vaccinia replicates completely within the cytoplasm of the host cell which implicates that the enzymes and factors necessary for transcription and replication are encoded in the viral genome. The virus is easy to grow in large quantities and is relatively safe to work with. Most mammalian cell lines are susceptible to infection because vaccinia virus has a wide host range. However, the efficiency of infection of different cell lines differs substantially.

The replication and assembly of vaccinia virus is very complex. The viral envelope fuses directly with the plasma membrane after binding to the host cell, releasing the cores into the cytoplasm (Doms *et al.*, 1990). Vaccinia is a dominant virus which means that it shuts down the host transcription and translation. Transcription of early genes begins one hour after-infection. The viral core contains all the enzymes needed for transcription of the early genes in addition messenger RNAs are capped, methylated and polyadenylated in the cytoplasm by viral enzymes. DNA replication starts at around two-three hours after infection. It occurs in the discrete regions of the cytoplasm near the Golgi complex called "viral factories". Naked viral DNA is not infectious because factors regulating the viral transcription are different from their host counterparts and are not interchangeable. After transcription of intermediate and late viral genes by different viral RNA polymerases, the complex process of viral assembly begins. Late genes code for structural proteins necessary for this process and they are driven by strong promoters. The viral core is inserted into a crescent-shaped, region between the endoplasmic reticulum and Golgi (Sodeik *et al.*, 1993) which fuses around the virus generating intracellular mature virus (IMV). The second wrapping membranes are derived from the trans-Golgi network (Schmelz *et al.*, 1994) and the resulting virus is called intracellular enveloped virus (IEV). Both membranes on the virion particles are probably associated with certain viral proteins which are localized either in the inter-

mediate compartment or in the trans-Golgi network. IEV can further fuse with the cytoplasmic membrane and extracellular enveloped viruses (EEV) are released into the extracellular space. Both forms of intracellular virus as well as extracellular virus are infectious.

SYSTEMS FOR PROTEIN EXPRESSION USING VACCINIA VIRUS

Vaccinia virus is the best studied member of the Poxviridae because it has a long history of being used as a vaccine against smallpox. Its complete nucleotide sequence is known (Goebel *et al.*, 1990) but its ancestry still remains a mystery. Definitely it is not the same virus that was used by Edward Jenner for vaccination against smallpox. It is likely that it is a hybrid between cowpox virus and a poxvirus which no longer exists in nature (probably a buffalo poxvirus). Whatever the origin of vaccinia virus, it is now relatively safe and has not been reported to produce severe illness. This fact in conjunction with the ability of vaccinia genome to accommodate up to 25 kb of foreign DNA and the presence of some nonessential viral genes which can be removed from the genome without a loss of viability in cell culture, resulted in the development of vaccinia expression system.

Expression of foreign genes using vaccinia virus is based on recombinant viruses constructed by insertion of foreign DNA into a nonessential vaccinia gene, usually the thymidine kinase (TK) gene.

Viral glycoprotein genes which are planned to be expressed in vaccinia system have to conform to some rules.Vaccinia transcription occurs in the cytoplasm and cannot make use of the host splicing machinery. Furthermore, it does not encode its own splicing machinery. Therefore, cloned DNA sequences must not contain introns. It was also found that a short sequence TTTTTNT is a signal for transcription termination of vaccinia early genes (Yuen & Moss, 1987). Foreign genes containing such sequences should be mutagenized by site-directed mutagenesis before insertion into vaccinia genome because otherwise trun-

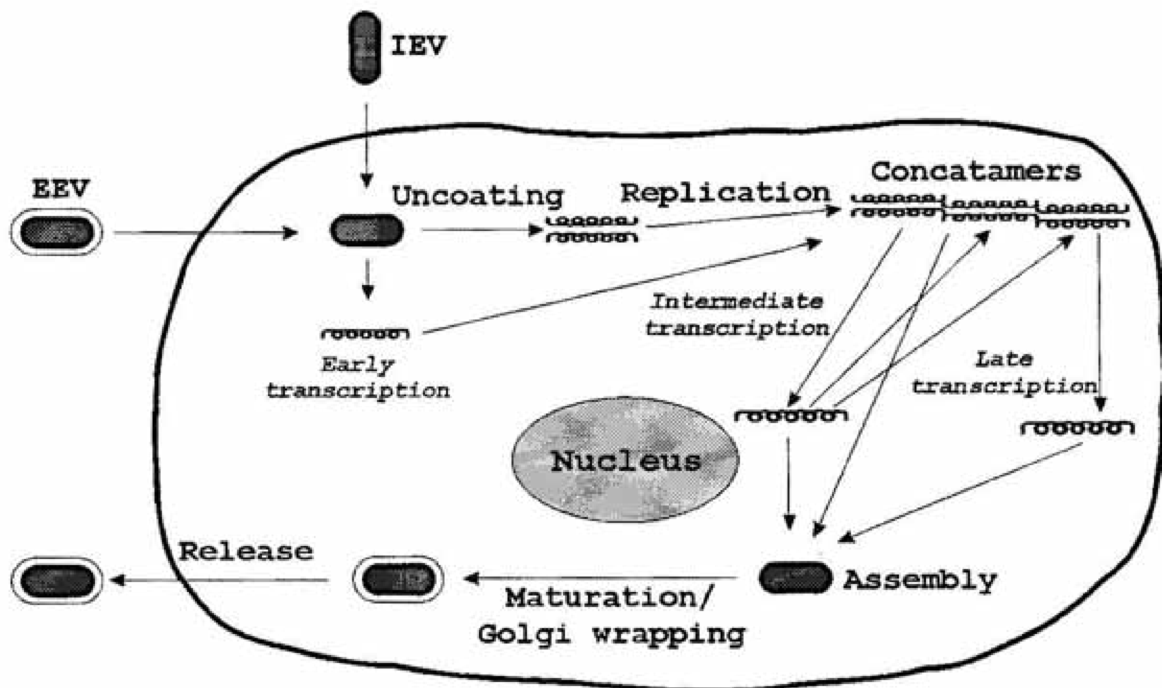

Figure 1. Infection cycle of vaccinia virus.

IEV, intracellular enveloped virus; EEV, extracellular enveloped virus.

cated proteins may be produced if the foreign gene was introduced under one of the early promoters. It has to be stressed that in this expression system only vaccinia promoters can be used. Other promoters, host cell or viral, are not recognized by the transcriptional machinery of vaccinia virus.

The insertion of foreign genes is usually performed by homologous recombination (Mackett *et al.*, 1982; Panicali & Paoletti, 1982). The most widely used system for insertion of genes employs plasmid transfer vectors containing vaccinia promoter, unique restriction enzyme sites for gene insertion and vaccinia virus DNA sequences flanking the non-essential gene site of insertion of a foreign gene (Mackett *et al.*, 1984). The procedure for recombinant vaccinia virus construction is outlined in Fig. 2. After introduction of a foreign gene by standard cloning procedures, the plasmid transfer vector is used to transfect mammalian cells. Simultaneously, the cells are infected with wild-type vaccinia virus. The virus enters the cells, is uncoated and viral genomic DNA becomes available for recombination with the transfer vector. Recombination is a rare event, so a suitable method of recombinant selection has to be used. If the insertion is in the thymidine kinase locus, then bromodeoxyuridine selection is usually used. Mutant cells which lack thymidine kinase (i.e. TK^-) will grow on 5-bromodeoxyuridine, a compound which can only be incorporated (and therefore kill cells) if a cell is TK^+. Derivatives of the transfer vector have been described which allow simultaneous introduction of the β-galactosidase gene into vaccinia genome in the same locus as the foreign gene (Chakrabarti *et al.*, 1985; Panicali *et al.*, 1986). These recombinants can be screened on the basis of the colour of plaques when a suitable substrate (e.g. X-gal) yielding blue product is added to the selection plates. By introducing neomycin phosphotransferase gene in the same way as the β-galactosidase gene, cells carrying recombinant virus become resistant to neomycin and can be easily selected on plates containing this antibiotic (Franke *et al.*, 1985).

Foreign genes can be also expressed in a hybrid system where bacteriophage T7 RNA polymerase and bacteriophage T7 promoters are utilized (Fuerst *et al.*, 1986; Fuerst *et al.*, 1987). This is a modification of an expression system widely used in bacteria. Its rationale is based on the very high specificity of bacteriophage T7 RNA polymerase for its own promoters and the very high efficiency of RNA synthesis by this polymerase. As depicted in Fig. 3, a recombinant vaccinia virus encoding T7 RNA polymerase is used to infect cells. The enzyme is expressed efficiently in the cytoplasm early after infection. Transfection of the same cells shortly after the infection with a plasmid carrying the foreign gene cloned behind bacteriophage T7 promoter results in the efficient expression of the encoded protein. It should be noted that a recombinant virus carrying foreign gene is not produced in this system.

The number of foreign genes that have been introduced into vaccinia virus is very large. One of the areas of the extensive use of vaccinia virus is the construction of potential live recombinant vaccines. Although a number of genes coding for glycoproteins of human viruses has been introduced into vaccinia virus, the application of these recombinants in human vaccination programs is not very likely because of the strong objections of medical authorities in many countries to the use of live recombinant viruses as vaccines. These objections do not apply to the use of vaccinia in veterinary practice. At least one vaccinia virus-based vaccine is currently used in many countries (Rupprecht *et al.*, 1986). This is the anti-rabies oral vaccine which is distributed from air over large areas in the form of food baits for foxes and racoon dogs. Recombinant vaccinia virus contains a gene coding for the very immunogenic glycoprotein G which in rabies virus coats the surface of virions. Numerous other veterinary vaccines based on recombinant vaccinia virus are being field-

tested and in the near future they are likely to be introduced as protective measures against viral diseases.

ADVANTAGES AND DISADVANTAGES OF VACCINIA VIRUS MEDIATED GENE EXPRESSION OF FOREIGN GENES

- Vaccinia virus mediated expresssion is relatively quick and efficient.
- Vaccinia has a wide host range, so most of the mammalian cell lines can be used.
- Coexpression of two or more foreign genes is possible either by introduction of these genes into a single virus or by mixed infection with two or more single gene recombinants.
- Vaccinia virus is a very good system for production of biologically active mammalian proteins. Glycosylation and other post-translational modifications necessary for activity are preserved. However, it should be noted that some minor changes in glycan chain profile with respect to the natural counterparts may be observed. Vaccinia shuts down completely most of the host functions which implicates that the levels of glycosyltransferases are not necessarily the same as in the uninfected host. This means that viral glycoproteins expressed in vac-

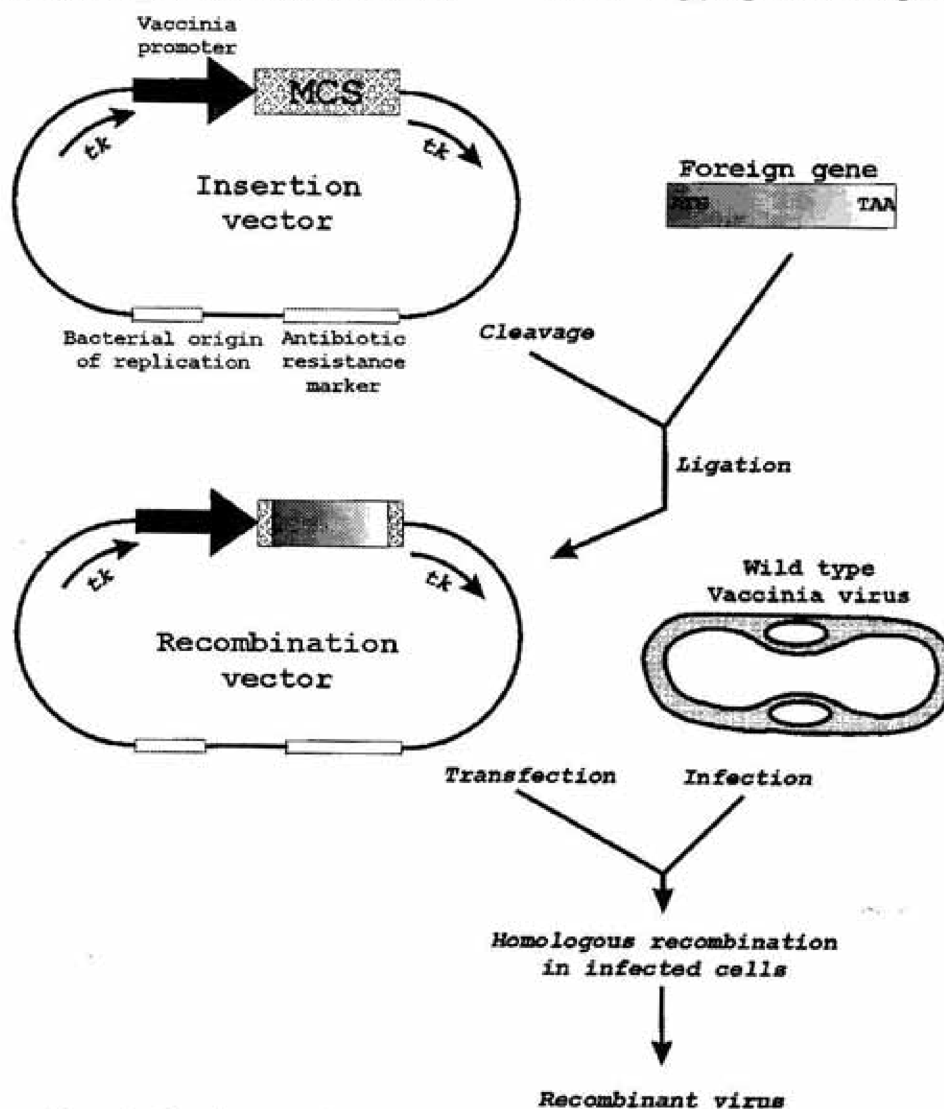

Figure 2. Insertion of a foreign gene into vaccinia virus vector.

MCS, multiple cloning site; *tk*, thymidine kinase gene.

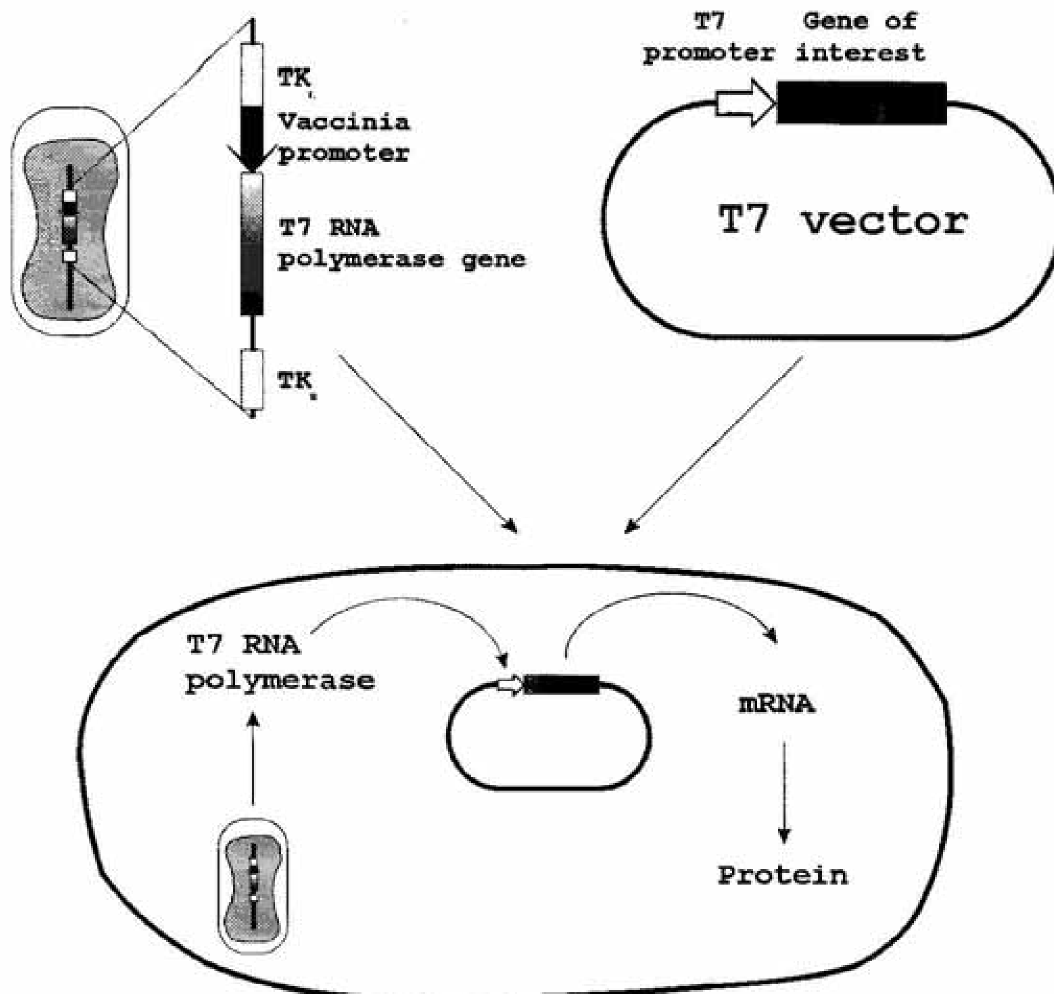

Figure 3. Schematic representation of indirect expression of a foreign gene using vaccinia/bacteriophage T7 RNA polymerase hybrid system.

cinia system, especially of these viruses which replicate within the cell without its disruption, undergo posttranslational modifications in a slightly different environment than those modified in natural conditions.

- Recombinant vaccinia viruses can be difficult and time consuming to produce. This may be sometimes due to the presence of T_5NT sequences in a foreign gene which lead to the premature transcription arrest.
- Even though vaccinia virus is relatively safe for laboratory workers, special guidelines for the use of handling vaccinia virus recombinants must be followed. This requires special instrumentation and restricted-access space for performing the experiments.

BIOLOGY OF BACULOVIRUSES

Baculoviruses infect arthropods and are not infectious to vertebrates and plants. They are a large group of double-stranded DNA viruses (over 500 species have been described); the majority have been isolated from a few insect orders: Lepidoptera, Diptera, Hymenoptera and Coleoptera. Individual baculoviruses usually have a narrow host range limited to a few closely related species. Virions consist of one or more nucleocapsids embedded in an envelope. Viral genome ranges in size from 80 to 200 kb. The most widely studied baculovirus is the *Autographa californica* nuclear polyhedrosis virus (AcNPV). Early work on AcNPV was directed towards the development of viral pesticides (reviewed in Wood & Granados,

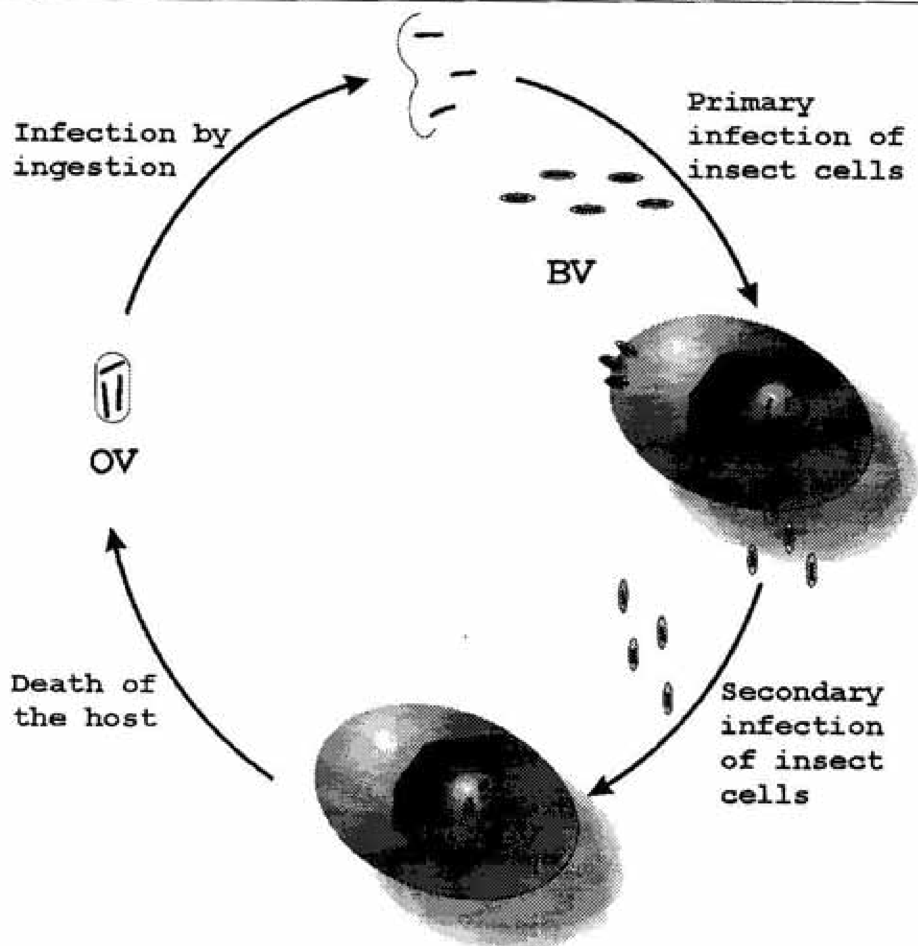

Figure 4. Natural life cycle of the baculovirus AcNPV.
BV, budded form of the virus; OV, occluded form of the virus.

1991). Current knowledge about the biology of this virus is largely due to the constant developments of baculovirus-based expression vectors.

The size of AcNPV genome is around 128 kb. The circular genome is complexed with a small basic protein of molecular mass around 7 kDa and this structure is protected by proteins forming the capsid. The genomic circular DNA is infectious in the naked form. Two morphologically distinct forms of the virus are produced at different times post-infection. Budded virus particles (BV) serve for the horizontal transmission of the virus to other tissues of the caterpillar body. Occluded viral particles (OV) are responsible for the survival of the virus in the environment and the spread of the virus from insect to insect. The occluded particles (polyhedra) contain many nucleocapsids surrounded by a matrix composed mainly of polyhedrin, the major structural protein. Polyhedra are stable and the protected virus can survive in the environment for more than twenty years. The natural cycle of infection by AcNPV in insect larvae is summarized in Fig. 4. Caterpillars ingest OV particles as contaminants of their food. The paracrystalline polyhedrin matrix is solubilized in the alkaline environment of the midgut of the larvae and the released virions enter midgut cells after fusion with membranes of the microvilli. The virions are uncoated and enter the nucleus where viral genes are expressed in a strictly controlled manner. Four phases of transcription are recognized: immediate early, delayed early, late and very late. Immediate early genes are transactivated by host transcription factors and viral proteins are not necessary at this stage. Transcription of delayed early genes requires acti-

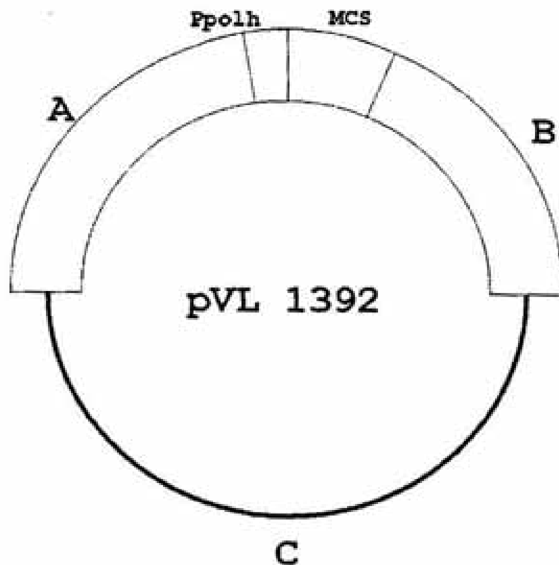

Figure 5. Schematic representation of a typical baculovirus transfer vector.

MCS, multiple cloning site; Ppolh, polyhedrin promoter; A and B, DNA sequences flanking the polyhedrin gene; C, bacterial replicon of a multicopy plasmid.

vation by products of immediate early genes. Initiation of transcription occurs within highly conserved promoter elements: CGT for early genes and TAAG for late genes which are usually located 30–90 bp upstream from the ATG start codon (Nissen & Friesen, 1989; Dickson & Friesen, 1991). The delayed early phase is followed by the synthesis of DNA and the late gene products of the virus (6–20 h post-infection). In the late phase nucleocapsid structural proteins are synthesized, including glycoprotein gp67 playing a crucial role in the horizontal infection by budded virus which is released from membranes 10–24 h post-infection (Whitford *et al.*, 1989). The very late or occlusion phase begins around 20 h post-infection. During this phase the production of infectious BV is greatly reduced. Nucleocapsids interact with nuclear membranes and eventually become enveloped usually in groups of a few particles (Fraser, 1986). The envelopment of the nucleocapsids appears to be the essential primary step in the process of occlusion of nucleocapsids by the very late protein – polyhedrin. The occlusion continues until the nucleus becomes filled with occlusion bodies. Typically around 30 polyhedra are seen within the nucleus. Around 10^{10} polyhedra are produced per late instar larva before death, which may account for over 30% of the dry weight of a larva (Miller *et al.*, 1983). As occlusion proceeds, fibrillar structures begin to accumulate in the cell nucleus (sometimess also in the cytoplasm). These structures are composed mostly of a single polypeptide p10 which is a very late protein (Van Der Wilk *et al.*, 1987). The function of the fibrillar structures is not clear but they may play a role in the controlled cellular disintegration in caterpillars (Williams *et al.*, 1989; Van Oers *et al.*, 1994). In the terminal stages of infection the caterpillar liquifies and releases polyhedra which can infect other insects.

SYSTEMS FOR GENE EXPRESSION USING BACULOVIRUS

Baculovirus genome is large, so the insertion of a foreign gene by direct cloning into a defined locus is very difficult. Recombinant baculoviruses are, therefore, usually constructed in two steps. Initially, a heterologous gene is introduced into a baculovirus transfer vector. A typical transfer vector is shown schematically in Fig. 5. It consists of a bacterial replicon of a multicopy plasmid, a selection marker gene, promoter and terminator regions along with flanking baculovirus sequences from a non-essential locus, and a multiple cloning site (or a single unique restriction site) downstream from a viral promoter. Usually the promoters and the flanking DNA originate from one of the late genes: polyhedrin or p10 gene. The baculovirus transfer vector containing foreign DNA, and genomic viral DNA are then introduced into insect cells where they recombine yielding recombinant virus with an integrated heterologous gene (Fig. 6). Polyhedrin and p10 pro-

moters are very strong promoters, so a high level of protein synthesis in insect cells is to be expected. The selection of recombinants by traditional methods was very tedious. The classical method of Summers and Smith (1987) takes advantage of different morphology of occlusion-negative and occlusion-positive baculovirus plaques. Plaques originating from viruses which have insertions in the polyhedrin gene appear less refractile than the polyhedrin-positive plaques and can be distinguished under a good quality microscope or even by naked eye. It requires, however, considerable experience to identify the recombinant plaques. Therefore, it is not surprising that many modifications of this procedure have been described. One of the first was the selection by dot blot hybridization. A probe is constructed from the cloned gene by introduction of radioactive atoms into the probe DNA or by using one of the non-isotopic labels (usually digoxigenin or biotin). The method works well but is time consuming.

A major breakthrough in the selection of recombinants was the construction of transfer vectors which allowed the introduction of a reporter gene along with a cloned gene into baculovirus genome. The reporter gene, most often *lacZ*, is usually inserted in the opposite direction to the cloned gene and it is under control of one of the weak baculovirus promoters (Vialard *et al.*, 1990; Zuidema *et al.*, 1990, Zhao *et al.*, 1991). The reporter gene is introduced into baculovirus along with the foreign gene, therefore the addition of a chromogenic substrate for β-galactosidase (e.g. X-gal) to the selection plates yields colored plaques (blue in the case of X-gal) which originate only from recombinant viruses.

Linearization of baculovirus genome at one or more locations simplifies the construction of recombinant baculoviruses. Linear baculovirus DNA exhibits a greatly reduced infectivity compared with preparations of circular DNA. When a unique Bsu36I restriction site was introduced into AcNPV genome which allows linearization in the vicinity of the polyhedrin gene, recombinant viruses were obtained at a frequency of about 30% (Kitts *et al.*, 1990). It should be noted that recombination between linear genomic DNA and a transfer vector results in circularization of the genome. So, even though the titer of recombinants per transfection is similar to this

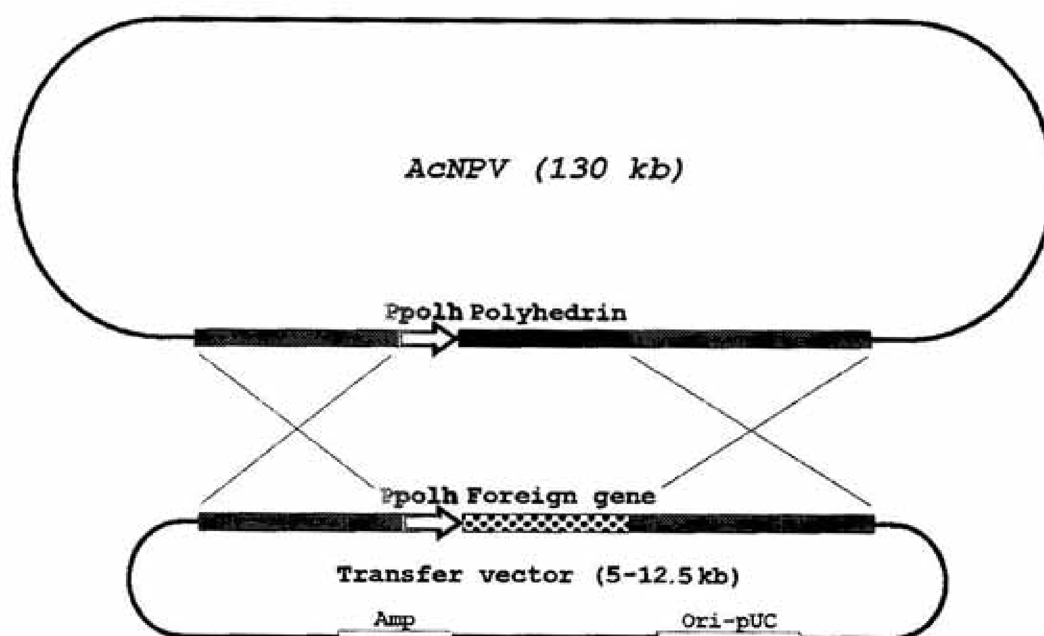

Figure 6. Generation of a recombinant baculovirus using circular genomic DNA.

Homologous recombination between the parental virus and the transfer vector leads to the formation of occlusion-negative recombinants at a frequency around 0.1%.

of normal cotransfections with circular genomic DNA, the percentage of recombinants is greatly increased because the background of non-recombinants originating from linear DNA is greatly reduced. The development of the above method increased the percentage of recombinant viruses to almost 100% (Kitts & Possee, 1993). The improved method of insertion of foreign genes into linear baculovirus is depicted in Fig. 7. The modified virus contains three Bsu36I restriction sites near the polyhedrin locus. One is in the *lacZ* gene under the control of the polyhedrin promoter, the second is in a non-essential gene upstream from the polyhedrin promoter, the third is in the essential gene ORF1629 downstream from the *lacZ* gene. The majority of the viable progeny (approaching 100%) have ORF1629 restored by homologous recombination between the transfer vector and the large Bsu36I fragment which contains the remainder of the viral genome.

A variety of novel strategies for construction of recombinant baculoviruses which do not rely on homologous recombination have been recently described. In the first method the construction of recombinant viruses relies on site-specific recombination between a transfer vector and a parent virus *in vitro* (Peakman *et al.*, 1992). Both the transfer vector and the parent virus were modified so that they contain a short segment (*lox*) recognized by the Cre enzyme of bacteriophage P1. When a transfer vector and a parent virus are incubated in the presence of the Cre enzyme, they recombine at the inserted *lox* sites. The second method of construction of recombinant baculoviruses is based on the use of a baculovirus shuttle vector that can propagate in yeast (Patel *et al.*, 1992). The shuttle vector, apart from the complete baculovirus genome, contains sequences ensuring stable replication in yeast (ARS and CEN) and two marker genes (*URA3* and *SUP4-o*). Recombinant shuttle vector is obtained by homologous recombination in yeast between the shuttle vector and the transfer vector. It can be purified over sucrose gradient and used to transfect insect cells. Even though recombinant viruses can be generated within two-three weeks, the need for good knowledge of yeast genetics and tedious shuttle vector purification prevented widespread use of the method. Currently, the first and the second method are superseded by a method for generating recombinant viruses in bacteria. This method, depicted in Fig. 8, is based on site-specific transposition of an expression cassette into a bacu-

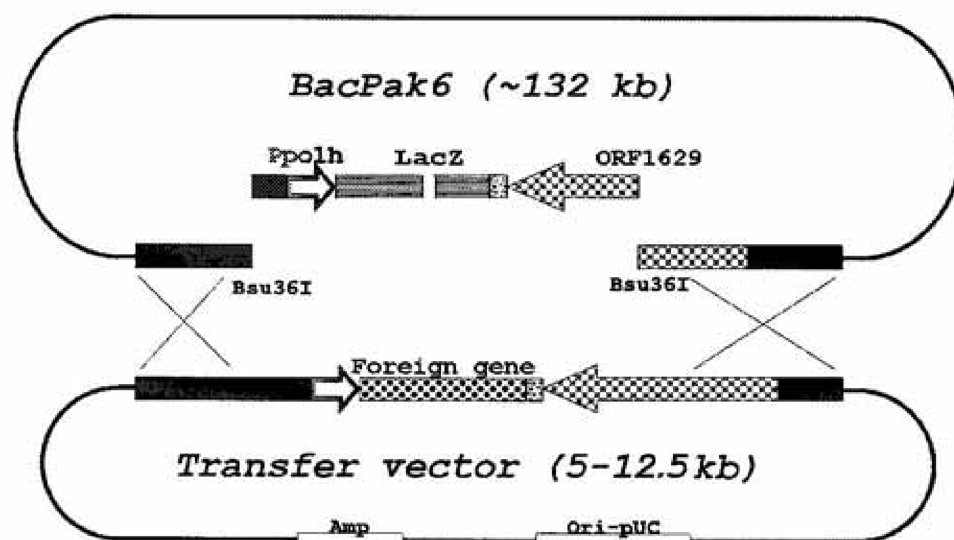

Figure 7. Generation of a recombinant baculovirus using parental genomic DNA linearized at three locations in and near the polyhedrin locus.

Homologous recombination between the linear genome and the transfer vector restores the essential viral gene ORF1629 after circularization.

lovirus shuttle vector propagated in bacteria (Luckow *et al.*, 1993). The shuttle vector (bacmid) contains the low-copy-number mini-F replicon, a kanamycin resistance gene and a DNA fragment coding for the LacZa peptide. At the N-terminus of the *lacZ* a short segment is inserted which contains the attachment site for the bacterial transposon Tn7. Bacmid propagates in *Escherichia coli* as a large plasmid conferring resistance to kanamycin and complementing a *lacZ* deletion present on the bacterial chromosome. Tn7 transposition functions are provided by a helper plasmid. The mini-Tn7 in a transfer vector contains the gentamycin resistance gene, a baculovirus promoter and a foreign gene. Transposition of the mini-Tn7 into the mini-attTn7 on the parent bacmid disrupts the expression of the *lacZa* and the bacterial colonies harbouring bacmid with a foreign gene are white instead of blue on X-gal plates. The recombinant bacmid DNA after simple isolation can be used to transfect insect cells where the baculovirus genome is replicated and recombinant baculoviruses are formed. Plaque purification is omitted here because each plaque should originate from a recombinant baculovirus. Working with bacteria is simple and fast, so it takes usually around two weeks to obtain a recombinant baculovirus.

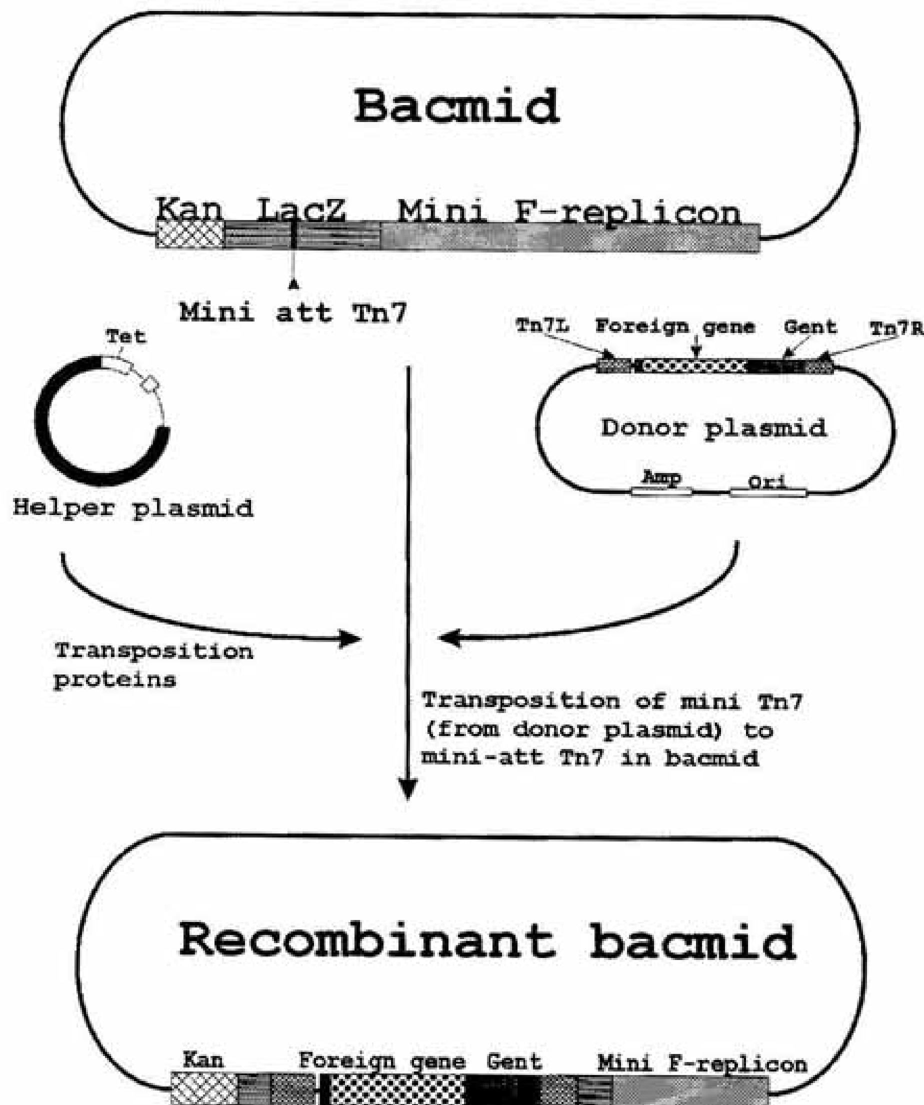

Figure 8. Generation of a recombinant baculovirus using bacmid, a baculovirus shuttle vector that replicates in bacteria.

The detailed description of the method is given in the text.

Recombinant baculoviruses are usually propagated in insect cell lines. Around 400 insect cell lines are available (Hink, 1989) but only a few support the growth of AcNPV. These lines were obtained from two parental organisms: *Spodoptera frugiperda* and *Trichoplusia ni*. The most widely used line is Sf9 which grows well in suspension (Vaughn *et al.*, 1977). N-glycan chains of glycoproteins produced in either *S. frugiperda* or *T. ni* cell lines have only mannose and fucose attached to the core *N*-acetylglucosamine and they lack complex oligosaccharides containing penultimate galactose and terminal sialic acid (Marz *et al.*, 1995). In addition, the levels of fucosyl transferase are different for cell lines originating from these two organisms. Glycans of glycoproteins produced in Sf9 cell lines are fucosylated to a much higher extent than in *T. ni* lines. The N-glycosylation pathway in lepidopteran cells can be extended by introducing functional mammalian processing enzymes to the insect cell lines. One of the approaches was to infect cells with baculoviruses expressing transferases along with the recombinant baculovirus expressing the desired glycoprotein (Wagner *et al.*, 1996; Jarvis & Finn, 1996). Recently, insect cells have been modified by cellular transformation to express constitutively a mammalian β1,4-galactosyltransferase gene and these transformed cells were able to add galactose to glycan chains of two foreign proteins (Hollister *et al.*, in the press).

Recombinant baculoviruses can be also used to infect caterpillars by ingestion of occluded virus or by injecting the budded virus into the hemocoel. The first method can be used for recombinants with healthy polyhedrin gene, so the foreign gene is usually introduced into p10 locus. The second method can be employed for recombinants with foreign genes in the polyhedrin locus. Expression of foreign genes in caterpillars is preferred for silkworm (*Bombyx mori*) larvae which in nature are infected by *B. mori* nuclear polyhedrosis virus (BmNPV) rather than for AcNPV hosts. Domesticated silkworms have been propagated for thousands of years by humans and some of their properties make them ideal organisms for the production of recombinant proteins. Caterpillars are large, grow very quickly, can be fed a synthetic diet and by automatic feeding machines. Hybrid viruses containing portions of both AcNPV and BmNPV have been generated that have expanded host range, so that the developments in AcNPV recombinant construction are applied to large scale glycoprotein production in silkworm larvae (Mori *et al.*, 1992; Croizier *et al.*, 1994).

ADVANTAGES AND DISADVANTAGES OF BACULOVIRUS MEDIATED GENE EXPRESSION OF FOREIGN GENES

- ◆ Very high levels of foreign genes are usually achieved compared with other eukaryotic expression systems.
- ◆ Baculoviruses have a host range limited to specific invertebrate species, so they are much safer to work with than mammalian viruses.
- ◆ Expression of hetero-protein complexes is possible by simultaneous infection of cells with more than one recombinant or by infection of cells with recombinants containing multiple-promoter expression cassettes. In this way incomplete or complete empty capsids of animal viruses can be constructed which may find use as potential vaccines (French *et al.*, 1990; Brown *et al.*, 1991; Sabara *et al.*, 1991; Roy, 1992).
- ◆ Baculovirus genome can accommodate large pieces (up to 20 kbp) of foreign DNA.
- ◆ Insertion of specific signal sequences in front of a foreign gene leads very often to the export of the gene product to the medium. By choosing synthetic media (Vaughn & Weiss, 1991) for supporting growth of insect cells, the purification of recombinant gene products can be then very simple.

- Apart from glycosylation, all other modifications of recombinant proteins in insect cells are the same, or very closely resemble the modifications of natural mammalian counterparts. As mentioned earlier, N-linked oligosaccharide processing capabilities in insect cells can be extended artificially; therefore, recent advances in the introduction of mammalian glycosyltransferases into insect cells may in the near future lead to "mammalian-like" glycan chains of baculovirus-expressed glycoproteins.
- If TAAG sequences are present within the heterologous gene, transcription may initiate within the cloned gene. If present in the antisense strand, the antisense RNAs may interfere with the expression of the heterologous gene.

CONCLUSIONS

The vaccinia and baculovirus expression systems have been used for production of hundreds of mammalian glycoproteins. These two systems are complementary and therefore the choice of the expression system should depend on the final application of the recombinant product. The fidelity of N-glycosylation is definitely much higher in the vaccinia virus expression system and this system should be employed in cases where glycan chain structure is critical for the activity. The ability to replicate in mammalian cells is also an advantage of the vaccinia virus system. When used as a recombinant vaccine, vaccinia virus harbouring a foreign gene will stimulate both humoral and cellular immune response in contrast to baculovirus-expressed antigen which will induce only humoral response. On the other hand, the level of recombinant glycoprotein synthesis is much higher in the baculovirus system. The simplicity and low cost of expression in the baculovirus system is another factor which very often favors the choice of this system in spite of some minor shortcomings.

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