Baculovirus for gene delivery to mammalian cells: Past, Present and Future

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Abstract

Baculovirus is an insect virus which has been used for more than thirty years for production of recombinant proteins in insect cells. However, baculovirus can also be harnessed for efficient gene delivery to mammalian cells if it is equipped with mammalian promoters. This technology is known as BacMam and has been used for gene delivery to immortalized cell lines, stem cells, and primary cells, as well as for gene delivery in animals. Baculovirus has unique features when compared to mammalian viruses. Besides the fact that it is replication-incompetent and does not integrate into the host genome, it has large capacity for foreign DNA. This capacity can for example be used to deliver multiple genes for reprogramming of stem cells, or for delivery of large homology constructs for genome editing. In this review, we provide a brief overview of baculovirus-based gene delivery and its recent applications in therapy and basic research. We also describe how baculovirus is manipulated for efficient transduction in mammalian cells and we highlight possible future improvements.

Key words: Baculovirus, gene delivery, mammalian cells.

1. Introduction

The baculovirus/insect cell system has been widely used in many academic and industrial laboratories for production of functional recombinant proteins (Kost et al., 2000; O'Reilly et al., 1994; Smith et al., 1983). Indeed, this system has some features that makes it unique as a protein expression system. Compared to bacteria it offers better folding of mammalian proteins (especially extracellular proteins), accurate posttranslational modifications, e.g., glycosylation, and the ability to produce protein complexes. Also, in comparison to mammalian expression systems it offers higher expression levels (Assenberg et al., 2013; Fernandes et al., 2013; van Oers et al., 2015). These high expression levels are based on the ability of the virus to take over infected cells in the late phase. In this phase, nearly all endogenous promoters are shut down and transcription mainly occurs from the viral polyhedrin and p10 promoters (Weyer and Possee, 1991). These two promoters are nowadays mainly used in commercial expression systems. So far, a tremendous number of properly folded proteins or protein complexes (e.g., antibodies (Guttieri and Liang, 2004), enzymes (Hodoniczky et al., 2005), hormones (Kim et al., 2005), kinases (Gao et al., 2005), membrane proteins (Trometer and Falson, 2010), multiprotein complexes (Berger et al., 2004), and virus like particles (Fernandes et al., 2013)) from different organisms have been produced using baculovirus/insect cell system. For example, Cervarix, a virus-like particle vaccine against human papillomavirus types 16 and 18 (from GSK), was produced using baculovirus/insect cells system. This product is approved by European Medicine Agency (EMA) and FDA (Harper et al., 2004). However, in the mid-1990s, new possibilities for the baculovirus system were identified. Two groups equipped baculovirus with mammalian promoters and efficiently transduced vertebrate cells (Boyce and Bucher, 1996; Hofmann et al., 1995). This system was called "BacMam" and has been used for many in vitro to in vivo applications. BacMam has interesting features for gene delivery that differentiate it from other viral and non-viral gene delivery approaches (Airenne et al., 2013; Kost et al., 2005; Uef, 2011; van Oers et al., 2015). Baculovirus is a non-integrating virus, with a large transgene capacity (>100kbp), and does not replicate

in mammalian cells (Mudgal et al., 2013). Also, baculovirus transduces a variety of cell types including both dividing and non-dividing cells, and shows a low cytotoxicity in vertebrate cells (Airenne et al., 2013). Emerging new applications in cell biology and drug development require gene delivery systems that have a high level of safety in delivery, flexibility in size of cargo, and simplicity in production. For example, gene therapy approaches in humans, of which there have been an increasing number in recent years, might be used successfully to treat cancer. For example, Kymriah™, a commercial preparation of T cells expressing a chimeric antigen receptor, calls for vehicles with a high level of safety and efficacy in gene delivery (Dunbar et al., 2018). Kymriah is based on a retrovirus, but an engineered baculovirus with improved features may fulfil these demands in future.

In this review, we first briefly describe the history of baculovirus biology. Then, important papers which used the BacMam system for delivery of either single or multiple gene to mammalian cells are highlighted. Next, we focus on recent in vitro, ex vivo and in vivo applications of the BacMam system in therapy and basic research (see (Mansouri and Berger, 2018) for a more comprehensive list of applications). Then, approaches for improvement of the virus are discussed. Finally, we highlight future directions toward an optimized baculovirus.

2. Baculovirus biology

Baculoviruses are a diverse group of DNA viruses capable of infecting hundreds of insect species. mainly lepidopterans (Chen et al., 2011). Baculovirus normally infects and ultimately kills insect cells (van Oers et al., 2015). Therefore, these viruses are also used as biological pesticides to protect crops from insects (Summers, 2006). Their genomes are circular double-stranded DNA, with a size of 80 to 180 kbp, and are packed in a rod-shaped nucleocapsid surrounded by a lipid envelop. Typically baculoviruses are 40-50 nm in diameter and 200-400 nm in length (Ihalainen et al., 2010). *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the best studied and most extensively used baculovirus (van Oers, 2011). It was initially identified in 1971, and the consensus sequence of the AcMNPV genome was published in 1982 (Vail et al., 1971; Vlak

and Smith, 1982). These pioneering studies were the prerequisite for the first AcMNPV-based baculovirus expression system (Smith et al., 1983). Thus, baculovirus discussed here refers to AcMNPV unless otherwise mentioned. AcMNPV is characterized by having two different virion cycle types, budded virions (BVs) and occlusion derived virus (ODV). BV bud from the cell membrane and spread infection within the host, whereas ODVs gain their envelope from the nuclear membrane and spread infection between hosts (Federici, 1997; Funk et al., 1997). In vitro expression systems work with the BV type. Exact mechanism and the receptor(s) on the host cell surface which mediate AcMNPV cell entry are not completely understood (Airenne et al., 2011). However, mammalian cell surface components like heparin sulfate, proteoglycans, phospholipids, and integrins are receptor candidates for AcMNPV (Airenne et al., 2013). Interaction between cell surface receptors and AcMNPV major envelope proteins, e.g., gp64, triggers attachment of the virus to the cell. After that, viruses are endocytosed and the associated change of pH inside early endosomes stimulates conformational change of gp64 which result in fusion of the viral envelope with the membrane of early endosomes. This fusion releases the nucleocapsid to the cytoplasm of host cells. There the nucleocapsid translocates to the nucleus through the nuclear pore complex and releases its genome within the nucleus (Airenne et al., 2011; Kataoka et al., 2012; Long et al., 2006) .

3. Baculovirus for gene delivery to mammalian cells

After successful baculovirus-based protein production in insect cells, the question was raised whether baculovirus can infect and subsequently deliver genes to mammalian cells. Earlier studies showed that baculovirus is able to enter non-target vertebrates (Gröner et al., 1984; Volkman and Goldsmith, 1983) and also some uncertain observations suggested baculovirus driven gene expression in mammalian cells (Carbonell et al., 1985; Carbonell and Miller, 1987). In 1995, Hofmann and colleagues used a baculovirus harboring a CMV-driven *LacZ* gene to express β -galactosidase in human and rabbit hepatocytes (Hofmann et al., 1995). In 1996, Boyce and colleagues also confirmed efficient baculovirus delivery

and expression of β -galactosidase under control of a RSV promoter in different mammalian cell types such as HepG2 and primary hepatocytes (Boyce and Bucher, 1996). These two pioneering studies attracted attention to baculovirus as a potential vector for gene delivery. Since then, many groups in academia and the pharmaceutical industry have used baculovirus as a robust gene delivery system for a wide variety of cells including cell lines, e.g., HEK293, COS7, CHO, and BHK, stem cells e.g., ESCs and iPSCs, and primary cells, e.g., HUVEC, rat embryo fibroblasts (REF), and neurons, (Condreay et al., 1999; Kost and Condreay, 2002; Mansouri et al., 2016). Here, we revisit some important points that should be considered when BacMam is chosen as gene delivery system. First, BacMam technology requires a mammalian promoter. A variety of promoters with different stringency and efficiency are available for this purpose (Qin et al., 2010). Tissue-specific promoters allow expression of the transgene only in desired cells within a mixture of cell populations or tissues (Zheng and Baum, 2008). Also, some common promoters (e.g., CMV) are either inactive or have minimal activity in some cell types, e.g., ESCs, (Sinn et al., 2017). Therefore, the mammalian promoter should be carefully chosen based on the target cell type. Second, it has been shown that baculovirus is able to transduce different mammalian cell types. However, baculovirus doesn't transduce all mammalian cells with the same efficiency. For example, a low level of transduction was reported in cells of hematopoietic origin (Schauber et al., 2004). However, there are strategies to improve tropism of the virus and to increase transduction efficiency (see the next part). Third, BacMam is considered to be a transient expression system. Nevertheless, addition of some elements, e.g., antibiotic resistance gene, transposon sequences, permits generation of stable cell lines from transduced cells (Condreay et al., 1999; Luo et al., 2012; Turunen et al., 2014). Fourth, it has been shown that addition of histone deacetylase inhibitors, e.g. sodium butyrate, can increase levels of gene expression in transduced mammalian cells (Sarkis et al., 2000). Although, this method is helpful for protein production in cells, it should be considered that these inhibitors usually have toxic effects on mammalian cells and can change the cellular behavior. Fifth, generation of baculovirus takes place

in insect cells at 27°C (Sung et al., 2014). Therefore, access to special cell culture equipment is required for baculovirus production.

Nowadays, production of baculovirus for BacMam can be done by introducing mammalian expression cassettes in commercially available vectors for insect cells. For example, pFastBac (Invitrogen) is one of these vectors. This vector also contains Tn7L/Tn7R sequences which are essential for transposition of the recombinant plasmid (carrying expression cassette) into the baculovirus genome (Bac-to-Bac, Invitrogen). Nevertheless, other published baculovirus systems are also suitable for this purpose. See (Lin et al., 2011) for an overview of available systems.

One of the most interesting features of baculovirus which gives it an advantage in comparison with other common viral vectors such as lentivirus and adeno-associated virus (AAV) is its large foreign gene capacity (a brief comparison between baculovirus and other commonly used viruses is provided in table 1). To make use of this possibility, we developed a baculovirus-based multigene delivery system for mammalian cells termed “MultiPrime”. Individual plasmids with independent expression cassettes are assembled by an in vitro cre/loxP recombination to overcome cloning problems associated with large constructs (see (Mansouri et al., 2016) for a detailed workflow). The same strategy was used earlier to assemble constructs for the production of protein complexes (Berger et al., 2004; Bieniossek et al., 2008; Fitzgerald et al., 2006). MultiPrime can efficiently transduce different cell lines, stem cells and primary cells both in vitro and in vivo. We showed that it is possible to deliver up to five genes simultaneously into a single cell, for example for reprogramming cells. Baculovirus also allows delivery of homology constructs that are necessary for genome editing (Mansouri et al., 2017, 2016).

4. Cell culture applications of BacMam

BacMam can be used for a wide variety of applications (Figure 1). Since generation of stable cell lines for cell-based assays is usually time consuming, tedious, or not possible, BacMam can act as a quick and efficient system to modify cells for cell

biological assays or drug screening (Ames et al., 2007). A commercial format of BacMam-based cell-labeling biomarkers (Organelle Lights™ or Cellular Lights™ reagents) is sold by Invitrogen. These reagents express fluorescent proteins which localize to different cell compartments, e.g., Golgi, ER. These markers are useful tools for high-content screening (HTC) in a fluorescent microscopy platform for analysis and detection of new drugs, because the virus vectors are less toxic and more efficient than transient transfection of the corresponding plasmid (Ames et al., 2007). MultiPrime allows an expansion of this strategy by simultaneous expression of multiple cellular markers. We expressed three different fluorescently tagged RabGTPases (Rab5, Rab7 and Rab11). These labeled RabGTPases localized to different cell compartments and allowed us to quantitatively monitor trafficking of receptors, e.g., EGFR, upon activation with a labeled EGF, in real time (Mansouri et al., 2016). Another example of utilizing BacMam for drug discovery is the Transfluor assay, an image-based assay that allows monitoring of GFP-arrestin recruitment to GPCRs (Hudson et al., 2006). More recently, Wright et al. developed a cell-based thallium flux assay, using baculovirus to deliver the TASK3 potassium receptor in order to find a TASK3 activator. In a library of 1000 compounds they could find Terbinafine, which specifically activates TASK3 (Wright et al., 2017).

The BacMam system is also a fast and reliable system for large-scale production of recombinant products in mammalian cells. Examples include proteins which are used in basic research such as structural biology (Goehring et al., 2014) or for the production of lentivirus therapeutic vectors (Lesch et al., 2011). It is possible to equip baculovirus with a dual-host specific promoter (fusion of p10 and CMV) for production of recombinant antibodies not only in mammalian cells but also in insect cells (Mansouri et al., 2016; Philipps et al., 2008).

Cell reprogramming is another exciting development in current biology which has revolutionized stem cell biology (Shi et al., 2017). Overexpression of defined transcription factors can change the cell fate in committed cells and convert them to either stem cells (reprogramming) or even another somatic cell (direct reprogramming) (Takahashi and Yamanaka, 2016). Here, it is desirable to use a

viral vector such as baculovirus, that can ensure a high level of reprogramming without integration into the host genome (Shao and Wu, 2010). For example, Takata and colleagues used the BacMam system for reprogramming of mouse embryonic fibroblasts (MEFs) to iPSCs using forced expression of Yamanaka's factors (Takata et al., 2011). They used a single ORF encoding four transcription factors (Oct4, Klf4, Sox2 and c-Myc) under control of the CAG promoter. Similarly, direct reprogramming of MEFs to induced neurons (iN) is also possible by expressing Ascl1, Brn2, and Myt1L from a single virus with independent expression cassettes (Mansouri et al., 2016). Phang and colleagues, in a more precise way, have used zinc finger nuclease technology and baculoviral vectors for site-specific integration of Yamanaka's factors into the AAVS1 locus in human chromosome 19 (Phang et al., 2013).

Genome editing involves DNA sequence-specific modification using targeted nucleases in almost any organism (Hsu et al., 2014). To date, genome editing can be done using programmable nucleases including meganuclease, zinc fingers, TALENs, and CRISPR/Cas9 (Cox et al., 2015). Targeted nucleases enable the user to generate desired alterations (mutation, deletion, or insertion) by introducing double-stranded breaks to activate host DNA repair pathways. Here, delivery of all the components for efficient and precise genome editing is a necessity. Accordingly, there is recent evidence that baculovirus allows delivery of genome editing components in a safe and efficient manner. For example, Lei and colleagues used baculovirus carrying zinc finger nucleases and donor template for disruption (knock-out) of the CCR5 gene in hESCs (Lei et al., 2011). They obtained 5% genome editing in hES cells and a higher rate (10%) in human cell lines. Our group also developed a MultiPrime vector harboring gRNA, Cas9 and homology construct in a single baculovirus (Mansouri et al., 2017). We achieved a higher rate of EGFP insertion (knock-in) to the endogenous the HMGA-I locus in HEK293, HUVEC and hiPSCs using baculovirus compared to transfection with the corresponding plasmid. More recently, Hindriksen et al. also showed that baculoviruses are suitable for genome editing. They knocked out components of

the chromosomal passenger complex (CPC) and tagged the CPC recruitment factor with EYFP (Hindriksen et al., 2017).

5. In vivo applications for baculoviruses

The inability of baculovirus to replicate and integrate in mammalian cells makes this virus attractive as a safe viral vector for in vivo gene delivery. However, there are some challenges which need to be solved before undertaking in vivo applications. First, baculovirus doesn't diffuse within injected tissue. For example, Heikura and colleagues have used baculovirus for expression of β -galactosidase and VEGF-D^{ΔNΔC} in skeletal muscle of an ischemic rabbit model. However, transduced cells were detected only in the proximity of the needle track (Heikura et al., 2012). Second, baculovirus can be inactivated quickly by complement and the immune system. There are some strategies which can partly protect baculovirus from being neutralized by the immune system (Kaikkonen et al., 2011, 2010). We divided these strategies to six parts. First, baculovirus can be used for gene delivery in animal models that have a suppressed complement system. For example, it has been shown that injection of baculovirus into the liver parenchyma of C5-deficient mice leads to some transduced hepatocytes surrounding the injected area (Hofmann et al., 1998). Second, applying complement inactivators, such as complement receptor type 1 (sCR1) or cobra venom factor, prior to in vivo delivery of baculovirus can increase the transduction rate (O'Reilly et al., 1994; Sarkis et al., 2000). Third, virus engineering through expression of complement interfering factors, e.g. DAF, factor H, and C4b-binding protein, on the virus envelop can protect baculovirus from immune system attack (Kaikkonen et al., 2011). Fourth, baculovirus can be used for in vivo gene delivery in places which are not exposed to the immune system and are called "immune privileged sites", for example in the eye, brain and testis. Some attempts have demonstrated successful baculovirus-mediated gene delivery to retina, brain and testis in animal models (Haeseleer et al., 2001; Sarkis et al., 2000; Tani et al., 2003). However, it was recently shown that BacMam transduction in eye is less efficient than transduction with AAV, lentivirus and adenoviruses (Kalesnykas et al., 2017). Fifth,

in most of the studies AcMNPV is used for in vivo gene delivery. However, Liu and colleagues, have recently shown that *Bombyx mori* nucleopolyhedrovirus (BmNPV) is more resistant to complement inactivation than is AcMNPV both in vivo and in cell culture (Liu et al., 2017). Therefore, it may be better to use BmNPV for in vivo gene delivery. Sixth, baculovirus can be used for ex vivo gene delivery. Instead of injection of baculovirus into the body, which induces an immune response, appropriate cells can be isolated from the body. After transduction with baculovirus in the laboratory environment and appropriate quality control, they can be transplanted back to the body. For example, Lin and colleagues have co-transduced bone marrow-derived mesenchymal stem cells with Bac-CV and Bac-CB baculoviruses expressing VEGF and BMP2, respectively. Transplantation of these cells to a rabbit model of osteochondral defect could ameliorate bone healing (Lin et al., 2010).

Since direct injection of baculovirus to the body induces immune system responses, baculovirus can be used as an immune system inducer (adjuvant) for vaccine development (Abe and Matsuura, 2010).

BacMam has also been extensively used in cancer research (Airenne et al., 2013, 2010; Chen et al., 2011; Makkonen et al., 2015; Rivera-Gonzalez et al., 2011; Uef, 2011). Indeed, baculovirus vectors expressing cancer-relevant genes such as tumor suppressor genes and apoptosis-inducing genes have been tested in vivo in animal models for treatment of glioblastoma, hepatoma, melanoma, lung cancer and many others (Wang and Balasundaram, 2010). Furthermore, baculovirus-transduced bone marrow derived dendritic cells (BMDCs), which were tested in a mouse model in an ex vivo approach, could significantly suppress lung cancer (Suzuki et al., 2010).

6. Baculovirus engineering

Baculovirus did not evolve to serve as a gene delivery tool for mammalian cells. Thus, transduction efficiency, cell type specificity, and genome stability are major issues (Buchholz et al., 2015; Kaikkonen et al., 2011) (Figure 2). Transduction efficiency of baculovirus can be improved through genetic or chemical approaches.

Since the membrane in mammalian cells has a negative charge, baculovirus treatment with a positively charged chemical reagent such as polyethylenimine (PEI) prior to transduction can increase electrostatic interactions between host membrane and the virus. Thus, the final output will be an improvement in transduction efficiency in vitro and in vivo (Yang et al., 2009). Another chemical modification on baculovirus which can enhance its transduction efficiency is derivitization with poly ethylene glycol (PEG). For example, it was shown that PEGylation of baculovirus can improve transduction efficiency both in cultured cells, e.g., HEK293 and HepG2, and in vivo e.g., brain and lung, (Kim et al., 2006). More elegantly, the surface of the virus can be modified during its production by coexpressing surface proteins. (Buchholz et al., 2015; Kaikkonen et al., 2011). Expression of proteins like vesicular stomatitis virus glycoprotein (VSVG) (Barsoum et al., 1997), influenza virus neuraminidase (Borg et al., 2004), and F protein from *Helicoverpa armigera* (Mao et al., 2006) with an cells specific promoter, e.g., p10 or polyhedrin, leads to their incorporation into the baculovirus envelope. It was shown that VSVG increases both transduction efficiency and cell tropism of the virus. There are potent peptides (e.g., RGD motif (Matilainen et al., 2006)) which are able to enhance transduction and tropism but they cannot be directly expressed on the cell surface because they are too short. These peptides need a fusion partner on the baculovirus surface (Borg et al., 2004). The major envelope glycoprotein of baculovirus is gp64 (Grabherr et al., 2001; Makela et al., 2010). This surface protein provides a compatible fusion partner for display of foreign peptides on the viral capsid. For example, peptides can be displayed as amino-terminal fusions on a second gene copy of gp64 or a truncated version thereof (Boublik et al., 1995; Grabherr et al., 1997). In addition, gp64 orthologues such as VSVG can also act as fusion partners (Mangor et al., 2001). It has been shown that engineered baculovirus with cell-type specific peptides enhance gene delivery (Buchholz et al., 2015). For example, a short peptide motif from gp350/220 of Epstein–Barr virus, which naturally infects B cells, was displayed as gp64 fusion peptide on the baculovirus envelope (Mangor et al.,

2001). These engineered viruses show increased transduction efficiency in B cell lines.

Directed evolution is a mimetic of the natural evolution process (genetic diversification and selection) for selection of improved mutant forms of biomolecules (Kotterman and Schaffer, 2014). Directed evolution is very well established for AAV and lentiviruses, and some improved versions of AAV are currently in clinical trials (Kotterman and Schaffer, 2014). However, this method is not frequently used for baculovirus engineering to our knowledge. Nevertheless, there are some studies that show directed evolution of gp64 can increase transduction efficiency in mammalian cells. For example, Sinn and colleagues developed a new mutant of gp64 with two mutations selected through directed evolution. They also pseudotyped lentivirus with either mutant or wildtype of gp64, and demonstrated that the mutant version of lentivirus transduces primary human airway epithelial (HAE) cells more efficiently (Sinn et al., 2017).

Major issues of baculovirus prior to its use in therapeutic applications are for example genome stability, purification and storage of the virus. The genome tends to lose unnecessary genes during amplification, especially in the late phase (Pijlman et al., 2003, 2002). This can be addressed by using minimized baculovirus genomes with higher stability, e.g., SynBac (Vijayachandran et al., 2013). A de novo construction of a synthetic virus with essential elements or the outsourcing of genes on helper viruses/ plasmids as it is done for the generation of safe lentiviruses could be alternative. Since production of baculovirus takes place in insect media at low pH, purification and concentration of the virus is crucial, especially when dealing with sensitive primary cells. This step can be done either by ultracentrifugation (Sung et al., 2014) or ultrafiltration (Michalsky et al., 2010). However, both methods are time consuming and need special equipment. One suitable way for purification and concentration of baculovirus is tagging and purification using affinity chromatography. For example, Kaikkonen and colleagues displayed a biotin acceptor peptide (BAP) by fusion to gp64 or VSVG in the baculovirus envelop (Kaikkonen et al., 2008). They showed that purification of baculovirus can be done using magnetic particles and reached titers up to 10^{10}

pfu/ml. Also modification of the baculovirus genome can improve functionality of the baculovirus vectors. For example, insertion of a reporter gene such as EYFP under control of a late insect cell promoter (e.g. *polh*) can facilitate virus production (Berger et al., 2004). Infected cells turn yellow in the late phase, helping to identify the optimal time point to harvest the virus. Since these reporter genes are only active in insect cells, they do not interfere with gene delivery to mammalian cells. While short-term expression of baculovirus vectors is beneficial for some applications such as genome editing, long-term transgene expression is also desirable for other cases, for example CAR expression on T cells. Sustained gene expression can be achieved by combining transposon-based systems, e.g., sleeping-beauty or piggyback, with baculoviral vectors. For example, Turunen et al. developed a sleeping beauty-baculovirus (SB-BV) system by accommodating transposon repetitive elements on both sides of the expression cassettes along with coexpression of the SB100 transposase (Turunen et al., 2014). They observed transgene expression 63 days post transduction. In line with this, Luo et al showed that equipping the baculovirus vector with either Epstein-Barr virus expressed nuclear antigen 1 (EBNA1) or sleeping beauty can prolong expression of the transgene to 35 and 77 days, respectively. They also showed that the hybrid SB-BV system can be used for sustained expression of therapeutic agents, which led to hindering of tumor growth and finally increased life span in an animal model (Luo et al., 2012).

7. Conclusion

BacMam is a well-established method for gene delivery to mammalian cells. This system can be considered as a reliable vehicle for gene delivery, especially when safety, size and complexity of transgene(s) are an issue. However, the virus in its current form is not suitable for therapeutic applications. Genome stability, purification, cell type specificity, and immunogenicity are major issues. It is clear that baculovirus engineering can improve efficiency, specificity and also functionality of gene delivery to mammalian cells. In the last years, many solutions to these problems were developed. Nevertheless, although current baculovirus

technology is not yet at the same level as it is for lentivirus and AAV, the flexibility of the baculovirus will help us to solve these issues.

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Figure legends

Figure 1. Applications of a baculovirus. Baculovirus can be used as a gene delivery vehicle in a wide variety of applications such as modification and insertion of genes, reprogramming of somatic cells to stem cell or other types of somatic cells, production of recombinant proteins such as antibodies, development of vaccines such as influenza vaccine, production of T cells with Chimeric Antigen Receptor (CAR) for gene therapy against cancer, drug screening, and tissue engineering.

Figure 2. Baculovirus engineering. (Top) Typical workflow for the generation of a baculovirus for mammalian expression. One or several genes of interest (GOI) are cloned with appropriate mammalian promoters (e.g. CMV) and poly A signals (pA) into a shuttle plasmid. This shuttle plasmid is then transferred to the baculovirus genome by transposition or recombination, a baculovirus is generated in insect cells and this virus is then used to transduce mammalian cells. (Middle) Possibilities to adapt baculoviruses to different applications. (Bottom) Example of an improved baculovirus. The shuttle plasmid can contain Tn7 sites (black circles) for easier transfer into the bacmid (Bac-to-Bac principle) or a transposon system that allows integration into the host genome (yellow). The bacmid can also contain a fluorescent protein for monitoring virus production (p10-mCherry) for easier virus production.

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Table 1. Comparison between baculovirus and the other viruses for gene delivery to mammalian cells

	Baculovirus		Other viruses (e.g., retro-, lenti-, adeno-associated-, adenoviruses)
Pros	<ul style="list-style-type: none"> - Large cargo capacity - High transduction efficiency in both dividing and non-dividing - Non-integrating virus - BSL1 cell culture facility for virus handling 	Cons	<ul style="list-style-type: none"> - Small cargo capacity (usually below 10 kb) - Inability to transduce non-dividing cells (except retroviruses) - Integrating viruses (retro- and lentiviruses) - BSL2 cell culture facility for virus handling
Cons	<ul style="list-style-type: none"> - Long and time-consuming process for virus production - Transient gene expression - Need for extra equipment for insect cell cultures 	Pros	<ul style="list-style-type: none"> - Fast and simple process for virus production - Stable gene expression (except AAV and adenoviruses) - No need for extra equipment - FDA approved for gene delivery to human

Figure 1. Applications of baculovirus. Baculovirus can be used as a gene delivery vehicle in a wide variety of applications such as modification and insertion of genes, reprogramming of somatic cells to stem cell or other types of somatic cells, production of recombinant proteins such as antibodies, development of vaccines, such as influenza vaccine, production of T cells with Chimeric Antigen Receptor (CAR) for gene therapy against cancer, drug screening, and tissue engineering.

Figure 2. Baculovirus engineering. (Top) Typical workflow for the generation of abaculovirus for mammalian expression. One or several genes of interest (GOI) are cloned with appropriate mammalian promoters (e.g. CMV) and poly A signals (pA) into a shuttle plasmid. This shuttle plasmid is then transfer to the baculovirus genome by transposition or recombination, a baculovirus is generated in insect cells and this virus is then used to transduce mammalian cells. (Middle) Possibilities to adapt baculoviruses to different applications. (Bottom) Example of an improved baculovirus. The shuttle plasmid can contain Tn7 sites (black circles) for easier transfer into the bacmid (Bac-to-Bac principle) or a transposon system that allows integration into the host genome (yellow). The bacmid can also contain a fluorescent protein for monitoring virus production (p10-mCherry) for easier virus production.