

Baculoviral Vectors

Baculoviruses are a virus family which probably originated 400 to 450 million years ago and are ubiquitous in the modern environment (1). Apart from ancient Chinese literature, the earliest evidence of baculoviruses in Western literature can be traced to the sixteenth century by Marco Vida of Cremona describing gory liquefaction of silk worms (reviewed in (2)). Starting from the 1940's baculoviruses were used and studied widely as biopesticides in crop fields (3). In the 1930's a specific baculovirus from Finland was successfully introduced to Canada to abolish spruce sawfly, *Gilpinia hercyniae* (4, 5). Since the late 80's and 90's they have been utilized as production of complex eukaryotic proteins in insect cell cultures (6) and later on for viral display (7).

In 1985 it was discovered that baculovirus with suitable promoter was able to transduce mammalian cells (8), an observation confirmed not until 1995 (9). Even though baculoviruses are hindered by complement-system of blood (10), successful *ex vivo* experiments soon led to successful *in vivo* experiments in 2000 (11). Since then, there have been several publications using baculoviruses with various targets *in vitro* and *in vivo* (12, 13).

The most studied baculovirus is *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). This virus was originally isolated from lepidopteran Alfalfa looper and contains a 134 kbp genome with 154 open reading frames (14). The major capsid protein VP39 together with some minor proteins forms nucleocapsid (21 nm x 260 nm, (15) which encloses the DNA with p6.9

protein. The virus appears in two distinctive forms depending on the stage of its lifecycle; a single budded virus (BV) and occlusion particle containing multiple virions, called occlusion derived virus (ODV).

BV obtains its envelope from cell membrane and requires glycoprotein GP64 to be able to spread systemic infection. This protein is not found on ODV while several other proteins are only associated to ODV. Some differences are also seen in the lipid composition of the viral envelope (16).

Baculoviruses have several advantages as a gene therapy vector. Viruses have a long history with extensive studies on safety and viral structure (17). They can be easily produced in high titers (up to 10^{12} pfu/ml), easily manipulated and quickly produced without animal serum (18). Baculoviruses are not restricted to transduce dividing cells only, but can transduce G1/S arrested cells (19). Most importantly, since viruses are derived from insect host, they do not replicate in vertebrate cells, however there is a contradictory report on expression of baculoviral immediate-early genes (20). Still, the safety of the occluded viruses has been studied with various methods including intravenous, oral, intracerebral and intramuscular administrations in experimental animals and with feeding tests on voluntary human without any signs of toxicity (21, 22). Yet, very limited information is available on the effects of high dose of budded viruses *in vivo*.

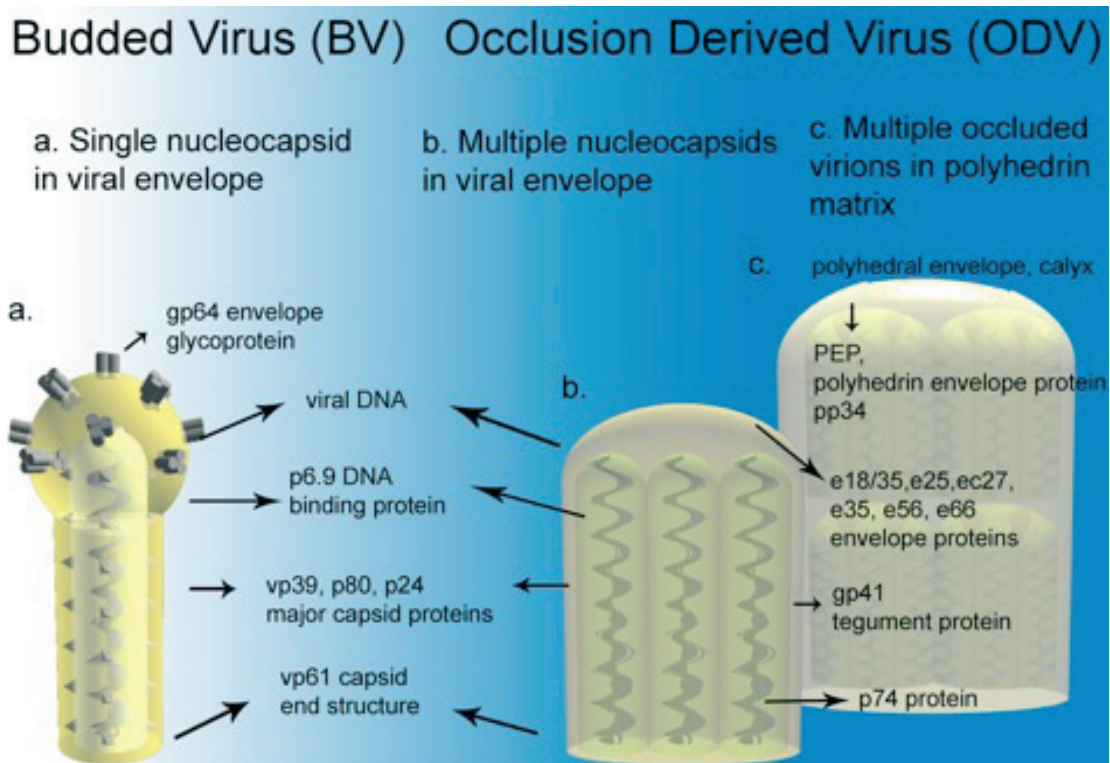
The rod-shape capsid enables high transgene capacity, without known limits (23, 24, 25). However, the drawbacks include the production of virus in insect cells which results in

the display of foreign glycoproteins, thus increasing possible immunogenic responses and inactivation by the blood complement system by classical pathway (10, 26). This hindrance has been avoided by using complement-protecting factors (26, 27, 28), avoiding exposure to the complement (11, 29) or using the virus in immunoprivileged areas such as the eye (30) and the brain (31, 32). Currently the baculovirus has produced most promising results in vivo in the CNS gene delivery, for example inhibiting glioma cell growth in an animal model showing higher transduction rate in glioma as compared to surrounding brain tissue (33). Additionally, it has been reported baculoviruses may be able to utilize axonal transport to cell bodies (34). While the later observation may be regarded as safety risk, together these studies

may promote focusing the research to brain and CNS.

As compared to adenoviruses, the overall transduction efficiency of baculovirus is somewhat lower and while baculoviruses are less cytotoxic as compared to adenoviruses (11, 31), the baculovirus envelope is of insect origin, possibly creating immunoresponse with repeated administrations as suggested by cytokine eliciting interaction with Kupffer cells (35). Additionally, the large genome of baculovirus shows signs on unstability, hindering the production of established clinical gene therapy vectors (36).

Some of these drawbacks related to baculovirus properties can be avoided by enhancing transduction efficiency by pseudotyping (37) or by inserting enhancing elements to viral transgene cassette (Mahonen AJ, sub-



Box 1 Baculovirus structural protein comparison between a) budded virus (BV), b) occlusion derived virus (ODV) and c) polyhedra embedded virions (OB) (modified from 48).

mitted) or by using histone deacetylase inhibitors (32, 38).

It is not currently known if the baculoviruses have a specific receptor for their attachment and cell entry. However there are reports on specific requirements for their transduction (19), involving heparin sulphate residues and electrostatic interactions. Additionally, interaction of Gp64 and cell surface phospholipids have been shown to play a role in the viral entry (39, 40). Interestingly, disruption of the cell-cell junctions with chelator EGTA has resulted to increased transduction efficiency (41) achieved also by transient calcium depletion (42). As it has been shown that basolateral surface is important for the baculoviral transduction, the loosening of cell-cell junctions might enable the entry from the basolateral side.

While the baculovirus receptor remains unknown, a large number of susceptible cell types suggests the viral tropism to be result from very universal interactions. However, when examining the difference between highly permissive and less permissive cell lines, it has been reported that the difference was in the presence or absence of baculovirus DNA in the nucleus (43), indicating that while the attachment might be universal, the later steps with nuclear entry and viral disassembly are likely to affect the baculovirus transduction and tropism (44). Interestingly, baculoviruses are one of the few viruses which carry their capsid to the nucleus (19), enabling the transport of therapeutic proteins to nucleus by using capsid display (44).

The transgene expression of baculovirus is transient, peaking levels for 2-5 days in vitro

(45) and from 3-5 days to one week in vivo (11, 31), but without complement lasting to nearly 200 days (28). However these results are with universal promoters and the transgene expression length and strength or tropism can be modified by using different promoters (33, 46, 47).

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