A BUG IN THE SYSTEM

Insect cell-based viral vector production offers advantages for efficiency and safety, even if it requires some fine-tuning to achieve PEAK PERFORMANCE

The fall armyworm's cells make great vector-production biofactories.

KOCHIEVMV/GETTY IMAGES

The idea of producing a medicine within insect cells destined for human use may sound odd, and the fall armyworm (*Spodoptera frugiperda*) is nobody's idea of a standard laboratory model. However, cells derived from these larval moths are easy to culture and can be readily infected with baculovirus — an insect pathogen that can be reprogrammed to churn out enormous amounts of adeno-associated virus (AAV) for use in gene therapy. Indeed, the first gene therapy to reach the clinic — Glybera, from Amsterdam-based uniQure — was produced from baculovirus-infected insect cells for both clinical testing and commercial release.

Baculovirus was already in widespread use for producing protein-based drugs when, in 2002, Robert Kotin and colleagues at the US National Heart, Lung, and Blood Institute first demonstrated its suitability for AAV manufacturing. They infected Sf9 cell lines — derived from the fall armyworm — with three different baculoviruses: two containing essential genes for AAV particle production (*rep* and *cap*), and one containing the transgene sequence intended for delivery (Figure 1a). In this manufacturing process, the baculoviruses play a dual role, functioning as the 'helper' virus normally required for replication, as well as the vehicle for AAV genetic material. In their initial demonstration, Kotin's team achieved levels of productivity comparable with existing AAV manufacturing approaches — on the order of 50,000 functional viral particles per cell.

The baculovirus production strategy offers a number of advantages. First, Sf9 cells can be cultivated at high densities as free-floating suspensions in large bioreactors with volumes of up to 200 litres, enabling far more efficient AAV production than adherent cell cultures. These cells can also be grown under serum-free conditions, which improves biosafety by eliminating the presence of potentially immunogenic or toxic animal-derived proteins. Furthermore, baculovirus cannot replicate in human cells, though it is still necessary to purify out any unwanted viral particles as a prelude to clinical use.

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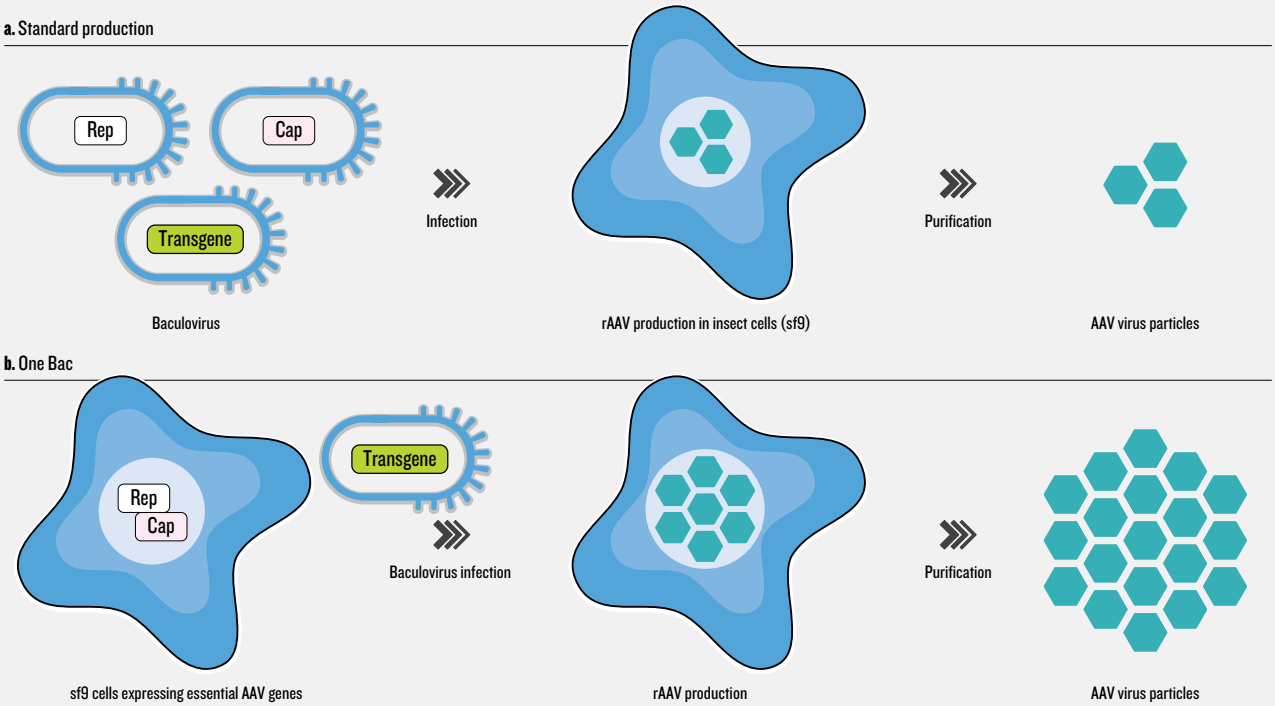
However, there are also significant limitations to baculovirus that require fine-tuning of the cultivation process. For example, the baculovirus genome can become unstable at high viral concentrations — and loss of the *rep* gene brings gene therapy vector production to a halt. This problem has been addressed to some extent by introducing genetic modifications to the baculovirus construct carrying this gene, which can improve genomic stability and prevent unwanted recombination.

The requirement for simultaneous infection of Sf9 cells with the three different baculovirus constructs also introduces complexity, and several groups have developed strategies intended to simplify the procedure. Perhaps the most notable is the BAC-to-AAV technology developed by Virovek, a company based

FIGURE 1: BACULOVIRUS-MEDIATED PRODUCTION OF AAV

(a) In conventional production systems, insect-derived cells (Sf9) are infected with a trio of viruses—two carrying the essential adeno-associated virus (AAV) genes *rep* and *cap*, and one containing the therapeutic transgene. This stimulates the Sf9 cells to produce infectious AAV particles. (b) The OneBac system simplifies this procedure by using Sf9 cells that have been genetically modified to carry *rep* and *cap*. These cells can produce large quantities of AAV after infection with a single baculovirus carrying the transgene.



in Hayward, California. This system employs specially-designed baculoviral constructs that are fine-tuned to ensure production of AAV component proteins at an optimal ratio for efficient viral assembly. This system also confers additional stability on the baculovirus genome, and gives rise to AAV vectors that achieve higher levels of infectivity — potentially enabling greater therapeutic efficacy when introduced into patients.

The OneBac platform, developed by Regine Heilbronn and colleagues at Charité Universitätsmedizin Berlin, employs Sf9 cells that have been genetically modified so that they already contain the *rep* and *cap* genes.

SF9 CELLS CAN BE CULTIVATED AT HIGH DENSITIES IN LARGE BIOREACTORS

Upon infection with a single baculovirus containing the full genome of AAV plus the therapeutic gene of interest, these cells produce AAV particles with an output that exceeds conventional baculovirus systems by an order of magnitude (Figure 1b). The first iteration of this system was unsuitable for clinical production because of a tendency to produce vector particles that contain unwanted baculovirus DNA sequences, introducing foreign proteins that could create safety issues in patients. Heilbronn's team has since devised a newer version, OneBac 2.0, which appears to eliminate this 'collateral packaging', although it remains, at present, unproven as a large-scale manufacturing technique.

Despite these technical challenges, the benefits of baculovirus-based manufacturing have made this approach a popular commercial alternative to the standard 'triple-transfection' AAV strategy. For example, BioMarin has used this manufacturing approach to support the clinical testing of valoctocogene roxaparvovec — one of the most advanced candidates in the gene therapy clinical pipeline. This AAV-based treatment for hemophilia A has demonstrated impressive performance in patients during phase I and II testing, and is now undergoing a pair of pivotal phase III trials. Accordingly, a growing number of companies are recognizing the possibility that this approach could help ameliorate some of the difficulties of AAV production. ■