Production of Adeno-associated viral vectors for gene therapy: studying vector genome production limitations in insect cells.

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Key words: Adeno-associated viral vectors, baculovirus, viral DNA replication, gene therapy vector.

Introduction. Gene therapy vectors derived from the adeno-associated virus (AAVv) are the first viral vectors approved for humans [1]. To accomplish the high vector titters required for their application, it is necessary to produce them at large scale as well as improving yields through more efficient bioprocesses. AAVv production in insect cells leads to bioactive particle (BP) yields similar to those of human cells providing a versatile and scalable platform. Nevertheless, empty capsid yields are ten-fold higher than BP yields suggesting limitations in AAV genome (AAVg) replication or packaging. The protein Rep78 is involved in AAVg replication and monomeric genome (mAAVg) -suitable for packaging- production and its concentration can affect both processes.
In this work, we studied the effect of Rep78 concentration on AAVg replication, rescue and stability to identify vector production limitations in insect cells.

Methods. High Five™ cells were infected at various multiplicities of infection (MOI) of the baculoviruses carrying the Rep78 protein gene (BacRep78) or the AAVg, which expresses the EGFP protein (BacGFP). Quantitative PCR (qPCR) was used to determine AAVg and baculovirus genome (Bacg) concentrations using the *egfp* and *p35* genes, respectively. AAVg rescue was evaluated by Southern blot. Rep78 relative concentration was determined by Western blot analysis.

Results. The effect of Rep78 on AAVg replication and mAAVg production is shown in figure 1. AAVg concentration increased 10-fold when expressing Rep78. BacRep78 MOI had no effect on AAVg maximal concentration ($5.47\pm1.10\times10^{11}$ AAVg/10^6 cells) but did affect the rescue from baculovirus DNA and the formation of mAAVg. Rep78 relative concentration, AAVg rescue from baculovirus DNA and mAAVg fraction across time were higher at 1 and 5 plaque forming units per cell (pfu/cell) of BacRep78 compared to 0.1 pfu/cell. Rep78 was more stable when expressed in cells containing the AAVg.

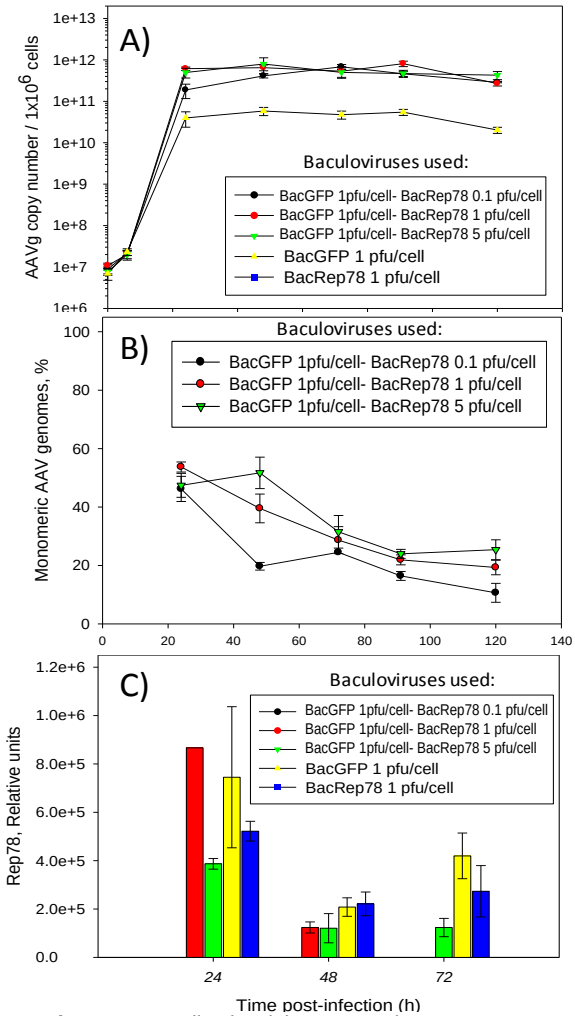

Fig.1 AAVg replication (A), monomeric genome resolution (B) and Rep78 relative concentration (C).

Conclusions. Maximal AAVg concentrations had the same magnitude than capsid yields, limiting AAVv production. Rep78 concentration affected mAAVg production and stability. Increasing Rep78 concentration could lead to higher monomeric genome concentration and AAVv yields.

Acknowledgements. The authors thank M. S. Moreno León, V. Hernández and R. Pastor for technical assistance. Financial support: PAPIIT-UNAM IT 200113.

References.

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