High titre BacMAM viruses improve the transduction efficiency of mammalian cells

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Introduction

•Transient production of proteins in mammalian cells is fundamental to many studies on gene function in health and disease.

•Many viral and plasmid vectors are available to enable the transfer of genes into mammalian cells, including baculovirus vectors.

•Baculoviruses are insect-specific viruses, which have been shown to transduce but not replicate in a wide variety of mammalian cells. So called BacMAM vectors rely on the integration of mammalian promoters to drive expression of the target genes with the designated cell line

•One disadvantage of the current BacMAM system is that relatively high multiplicities of infection (50-200+ virus particles per cell) are required for effective transduction

•This requires either concentration of the BacMAM virus (time-consuming/labour intensive) or the use of chemical enhancers (not desirable).

Objective

•We have constructed a novel BacMAM virus containing a mutation in an essential gene that results in consistent, very high titre budded virus so that high transduction efficiencies using 200+ particles per cell can be achieved without recourse to concentration of virus or addition of chemicals to enhance virus uptake.

Results

•The wild type virus (*Autographa californica* nucleopolyhedrovirus [AcMNPV]) genome comprises 155 genes.

•A point mutation in the coding region of one of these genes causes in a frame shift (Fig. 1), resulting in the introduction of a stop codon (TAG).

•This stop codon prevents most of the gene being expressed as a protein and interrupts its normal function.

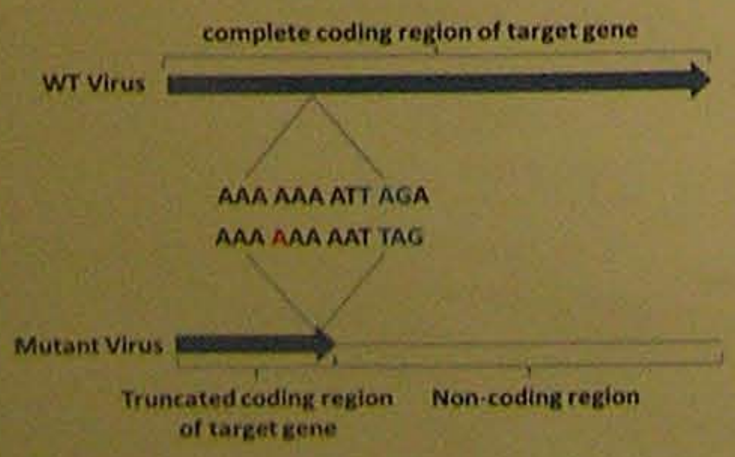

Figure 1 0 Schematic comparing the target ORFs coding region between WT and mutant virus (Ac-HT).

- The mutant virus (Ac-HT) appeared to replicate normally in cell culture and budded virus production was compared with AcMNPV.
- AcMNPV or Ac-HT virus were amplified in *Spodoptera frugiperda* (Sf9) and harvested after 5 days. Budded virus in the cell culture medium was titrated using a plaque assay
- Three separate experiments showed that Ac-HT virus consistently had higher titres than the AcMNPV (Fig. 2).

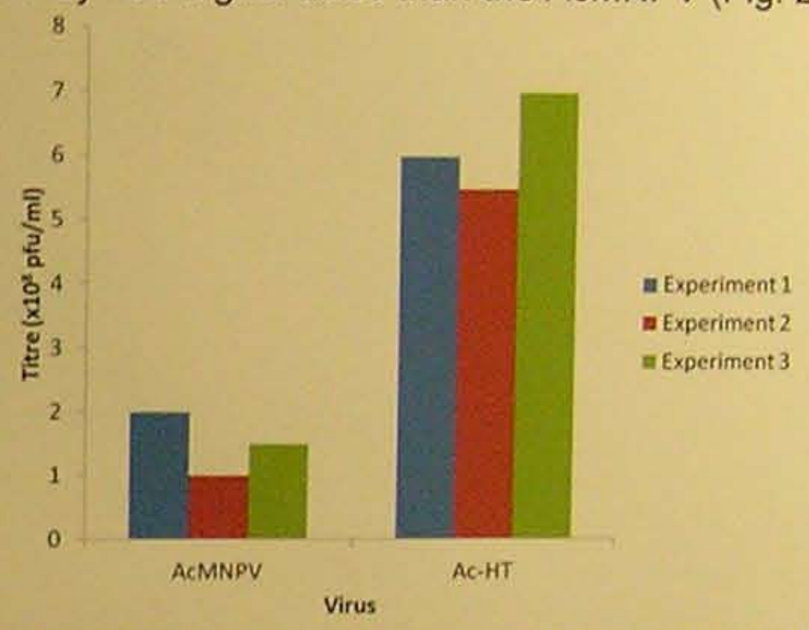

Figure 2: Budded virus titres of the Ac-HT virus compared to AcMNPV.

•Human Embryonic Kidney 293 (HEK293) cells were transduced with BacMAM virus expressing GFP at different MOIs. The transduced HEK293 cells were incubated for 48 hour post transduction and images were taken using a fluorescent microscope Figure 3.

•The images demonstrate that MOI 100 is not efficient enough to transduce a large percentage of the HEK293 cells, whereas at MOI 600 shows ~95% of the cells expressing GFP.

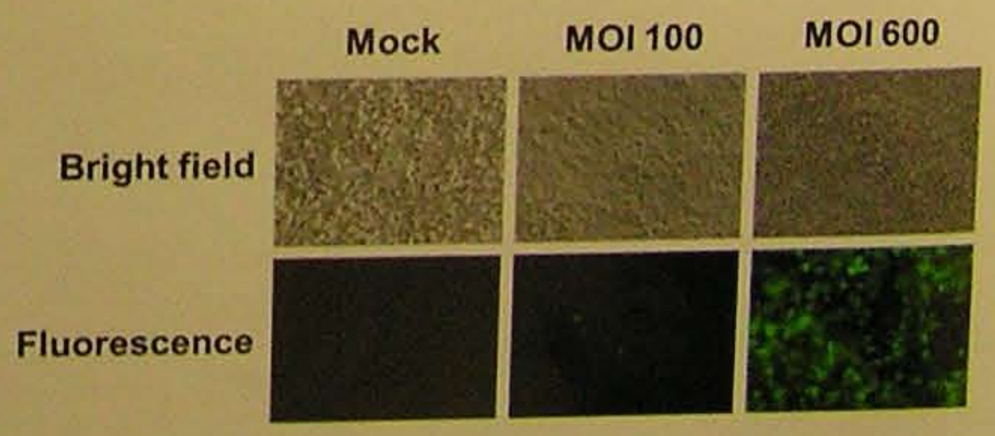

Figure 3: Bright field and fluorescent images of transduced HEK293 cells alongside mock transduced sample.

Conclusion/Future Work

- The results presented here demonstrate a single point insertion in the target gene resulted in an increase in the budded virus titre compared to wild type virus.
- This Ac-HT virus may help the development of BacMAM as a potential gene therapy vector.
- To incorporate this mutation in our patented *flash*BAC system to allow rapid generation of BacMAM viruses with this mutation.