Baculoviruses are a family of rod-shaped dsDNA viruses whose host range is restricted to intervertebrates, mainly insects. Among the baculoviruses, the most characterized species belonging to the nuclear polyhedrosis viruses (NPVs) have been widely used for production of recombinant proteins in insect cell cultures and insect larvae. More recently, since the discovery that these viruses can transduce efficiently many different mammalian cell types, the applications of the baculovirus technology have expanded to include their use as vectors for (i) production of foreign proteins in mammalian cells; (ii) gene therapy applications; and (iii) applications related to vaccine antigen delivery (Kost et al., 2005).

The use of baculoviruses for mammalian transduction offers a number of advantages as compared to mammalian virus-derived vectors. These include the ease of production in insect cell lines; their ability to package large fragments of foreign genetic material; their inability to function productively and produce progeny virus in cells other than those of their normal hosts; the lack of induced cytotoxicity even at the high multiplicity values used for efficient mammalian cell transduction; and the absence of pre-existing immunity against them in mammalian organisms.

We have previously reported on the development of baculovirus vectors derived from Bombyx mori nuclear polyhedrosis virus (BmNPV), which are capable of delivering efficiently mammalian expression cassettes into established and primary cultures of mammalian cells without affecting their molecular phenotype or their capacity to differentiate in vitro toward their normal phenotype (**Figure 1**; Kenoutis and al., 2006).

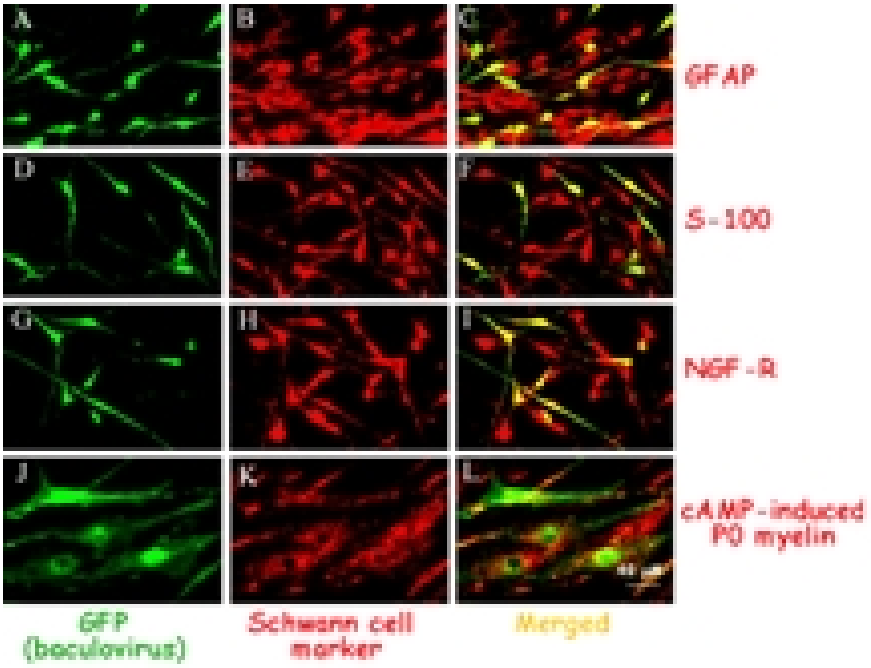


Figure 1 . Baculovirus-transduced Schwann cells retain a normal phenotype and (A to L)

Moreover, we have used recombinant BmNPV-based vectors to transduce HEK293 and primary Schwann cells (SCs) and express the therapeutic protein L1 in its native or a chimeric

form, L1-Fc. This study has shown that this protein can be successfully expressed in the transduced mammalian cells and is functional in various in vitro assays (**Figure 2**; Lavdas et al., 2010).

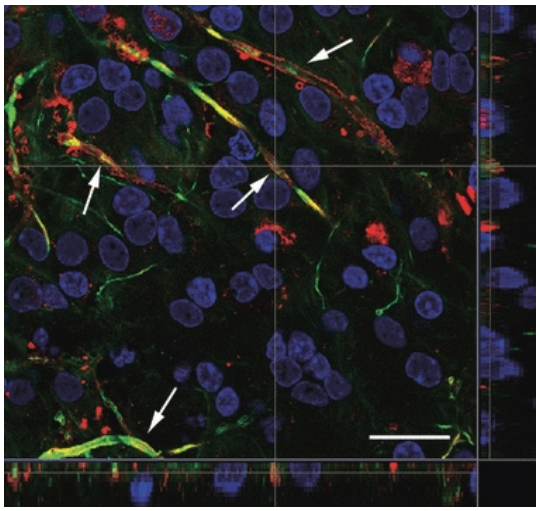


Figure 2. Baculovirus-transduced SCs seeded on cerebellar slices are able to myelinat

However, there are several issues that need to be addressed before engineered baculoviruses can be employed safely as gene therapy vectors in humans. One of them concerns the residual expression of baculovirus genes in infected mammalian cells. To address this issue, we produced infectious ie1-deficient viral particles lacking the gene encoding a major transcriptional regulator of the virus, IE1, which regulates multiple aspects of the baculovirus infection cycle, using insect host cells stably transformed with various IE1 expression constructs (Efrose et al., 2010). Our data demonstrated not only that such ie1 gene knockout viruses are unable to replicate and direct viral gene transcription in normal insect host cells to any appreciable degree but, also, that they are essentially devoid of the residual gene expression, which normally occurs in mammalian cells when the latter are transduced with wild type baculoviruses (**Figure 3**; Efrose et al., 2010).

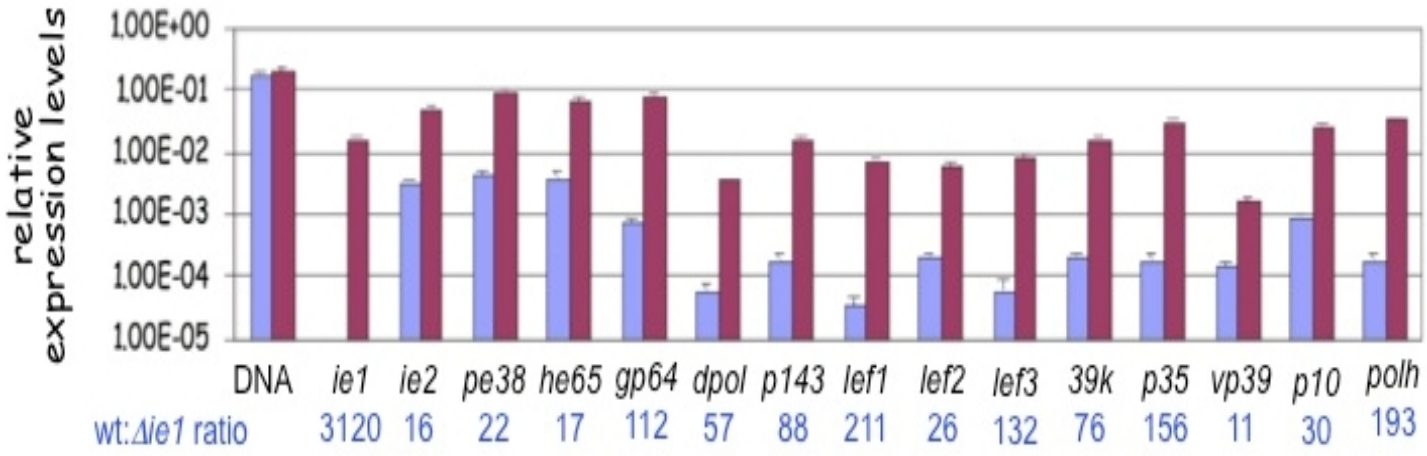
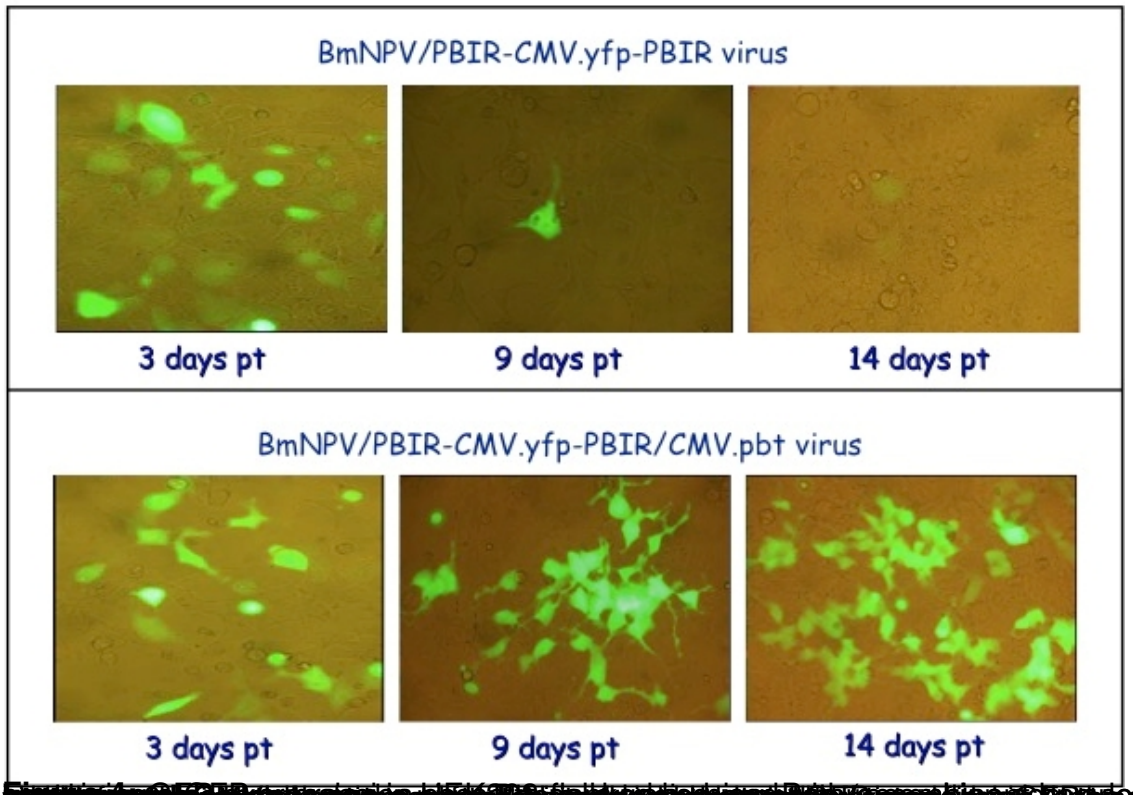


Figure 3: Accumulation patterns of viral gene transcripts in HEK293 cells transduced wit

The *ie1*-deficient baculoviruses are thus considered to be safer transduction vehicles for gene therapy applications due to reduced risks for physiological changes in infected cells and induction of cellular immune responses in individuals transplanted with *ex vivo* transduced cells.

Finally, to address a second drawback of the baculoviruses related to gene therapy applications, its presence as an episomal entity in the transduced cells, and achieve genomic integration and stable transformation of mammalian cells, a second generation of BmNPV-based recombinant baculoviruses employing the *piggyBac* transposition system was developed (unpublished data). The newly developed baculoviruses incorporate (i) a target *piggyBac* transposition cassette allowing the cloning of open reading frames for reporter or therapeutic protein expression under CMV promoter control and (ii) a mammalian *piggyBac* transposase expression cassette. Upon infection of mammalian cells, the latter causes transgene excision from the viral genome and integration into the genomes of recipient cells (**Figure 4**).



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