THE ROLE OF CELLULAR GROWTH FACTOR RECEPTORS IN MEDIATING GENE TRANSFER BY ADENO-ASSOCIATED VIRUS 2 VECTORS

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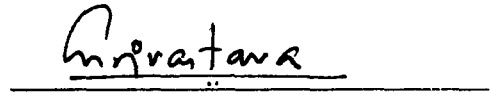

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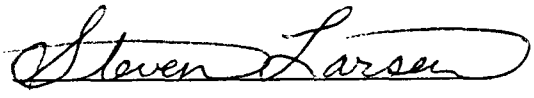

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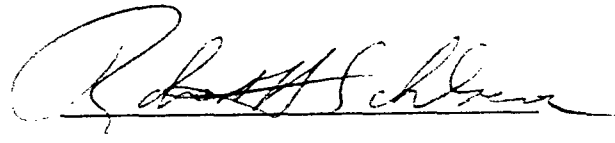

Robert Schloemer, Ph.D.

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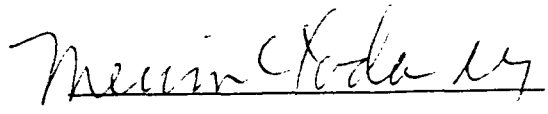

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THE ROLE OF CELLULAR GROWTH FACTOR RECEPTORS IN MEDIATING GENE TRANSFER BY ADENO-ASSOCIATED VIRUS 2 VECTORS

Adeno-associated virus 2 (AAV), a single-stranded DNA-containing, non-pathogenic human parvovirus, has gained attention as a potentially useful alternative to the more commonly used retroviral and adenoviral vectors for human gene therapy. However, the transduction efficiency of AAV vectors varies greatly in different cells and tissues *in vitro* and *in vivo*. In this study, the initial steps of transduction by AAV, that is virus entry into the host cell and the fate of the viral genome post entry were investigated. Although previous work has suggested that AAV uses the ubiquitously expressed cell surface heparan sulfate proteoglycan (HSPG) as a receptor, HSPG alone is insufficient for AAV infection. AAV also requires human fibroblast growth factor receptor 1 (FGFR1) as a co-receptor for successful viral entry into the host cell. Cells which do not express either HSPG or FGFR1 are resistant to infection by AAV and these cells can be successfully transduced by AAV vectors following stable co-transfections of HSPG and FGFR1 cDNAs. Furthermore, AAV infection can be abrogated by treatment of cells with basic fibroblast growth factor (bFGF). In addition to viral entry, investigation of AAV-mediated transgene expression has shown that epidermal

growth factor receptor protein tyrosine kinase (EGFR PTK) plays a crucial role in AAV transduction. Recent work from our laboratory has documented that a cellular tyrosine phosphoprotein, designated the single-stranded D sequence-binding protein (ssD-BP), plays an important role in AAV-mediated transgene expression and that a strong correlation exists between the phosphorylation state of the ssD-BP and AAV transduction efficiency *in vitro* and *in vivo*. The present studies demonstrate that EGFR PTK catalyzes the phosphorylation of the ssD-BP and that specific inhibition of EGFR PTK leads to a significant increase in AAV transduction efficiency. The identification of FGFR1 as a co-receptor for AAV and the role of EGFR PTK in AAV-mediated transgene expression should provide new insights not only into their roles in AAV-mediated transduction, but also in the successful use of AAV vectors in human gene therapy.

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