

Gene transfer to the nervous system using HSV vectors

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Summary

The natural history of Herpes simplex virus type 1 (HSV-1) infection in humans suggests its potential for development as a gene transfer vehicle suitable for nervous system applications. HSV-1 has a broad host range, does not require cell division for infection and gene expression and has evolved to persist in a life-long nonintegrated latent state without the expression of viral proteins or evidence of neurodegenerative disease in the immune competent host. The virus also has evolved a unique neuronal-specific promoter system that remains active during latency and fortuitously may be used to express therapeutic proteins without compromising the latent state. The establishment of latency also does not require the expression of viral lytic functions and thus removal of genes required for expression of the viral cascade of expressed products allows for safe vector design without the possibility of reactivation from latency. The HSV-1 genome is 152 Kb in length and of the 84 known genes (Roizman & Sears, 1996), approximately half are dispensable for virus replication in cell culture thereby providing considerable opportunity for introduction of foreign sequences. In this review, a brief overview of the biology of HSV relevant to vector design and progress in reducing virus cytotoxicity and its relevance to the level and duration of transgene expression is discussed. Methods for the expression of transgenes in the peripheral and central nervous system using the latency active promoters are described and strategies are suggested for potential applications to the treatment of neurodegenerative disease and cancer.

I. Introduction

The nervous system would appear to be a fertile site for the application of gene transfer for the treatment of disease where structural features of this tissue impede traditional pharmacologic therapy. These include the blood-brain-barrier which excludes macromolecules in the blood from entry into brain parenchyma and the cellular and regional specialization within the nervous system which may require the delivery of the therapeutic agent to restricted regions or to particular cells within those regions. Such impediments would be overcome by the direct transfer of a gene whose local expression resulted in the production of a macromolecule required for restoration of normal tissue function.

Several different classes of neurologic disease could theoretically be treated by gene transfer. The progression

of chronic neurodegenerative conditions (e.g. Alzheimer's disease, Parkinson's disease) might be ameliorated by the local production of neurotrophic factor(s) that prevent the degeneration of affected cells. Even though there is no evidence that these diseases are caused primarily by trophic factor deficiency, experimental evidence has shown that disease progression in animal models may be prevented by specific neurotrophins (e.g. nerve growth factor, glial derived neurotrophic factor) (Choi-Lundberg et al., 1997; Gash et al., 1996; Kearns and Gash, 1995; Tomac et al., 1995). An ideal vector for this application would constitutively produce low levels of the trophic factor in the region of interest for the life of the host. Intracranial malignancies of neural tissue origin or metastases from other tissues represent a second class of neurologic disease that might be treated by the transient local expression of therapeutic molecules. The therapy of brain tumors could

be enhanced by the expression within tumor cells either of immune modulators that attract tumor killing inflammatory cells and cells capable of differentiating into tumor-specific immune T cells or the transient expression of cytotoxic molecules (e.g. tumor necrosis factor) or enzymes (e.g. thymidine kinase) which locally activate anti-cancer drugs. Activated drugs such as ganciclovir and 5-fluoro-uracil can also kill dividing neighboring cells even without direct infection by cell to cell transmission or uptake of locally released drug. Multiple sclerosis, a relapsing remitting disease caused by immune mediated attack on central nervous system myelin, could be effectively treated by the transient expression of immunomodulatory cytokines that block the autoimmune attack, although prolonged expression of cytokine inhibitors would not likely be beneficial, requiring either repeat dosing of the vector or control of anti-cytokine gene expression by drug manipulation of therapeutic gene expression.

II. Gene transfer vectors for brain

Genes may be introduced into relevant cells of the nervous system directly *in vivo* or through transplantation of transduced cells (*ex vivo* approach). A crucial factor in gene replacement therapy is efficient transduction of the therapeutic gene into the target cell population and a wide variety of delivery vehicles including viral vectors, gold particle-DNA conjugate bombardment, direct injection of plasmid DNA, cationic liposomes alone or accompanied by fusion-promoting agents, and receptor-mediated endocytosis have been tested for gene delivery into neurons and glia. Viral vectors are widely and an efficient means of gene delivery. Replication defective herpes simplex virus (HSV), adenovirus (AV), human lentivirus-mouse retrovirus recombinants (HIV-MoMuLV) and adeno-associated viruses (AAV) have all been tested in animals. These viruses differ with respect to tropism, persistence as an episome versus integration into host chromosome, toxicity or antigenicity, longevity and level of gene expression, risk of tumorigenicity, maximum transduction capacity, and the ability to produce high titer viral stocks free of replicating virus contaminants. Retroviruses that require host cell division for their integration and expression can not be employed for *in vivo* gene transfer to the nervous system (Miller, 1992). On the other hand, viral vectors which either remain extrachromosomal, such as HSV-1 (Fink et al., 1992; Geller and Breakefield, 1988; Geller and Freese, 1990a) and AV (Davidson and Bohn, 1997; Mitani et al., 1995) or HIV recombinants (Naldini et al., 1996; Naldini et al., 1996) which are capable of integrating into the genomes of nondividing cells, are suitable for gene delivery to adult post-mitotic neurons. AAV has been shown to efficiently transduce particular neurons in brain, but it is unclear whether the viral genes

are integrated (Kaplitt et al., 1994). *Ex vivo* approaches consist of introducing the therapeutic gene into a cell population such as fetal or immortalized neurons, multipotent progenitor neural stem cells, adrenal chromaffin cells, glia, or fibroblasts that can be cultured *in vitro* and survive at or migrate to the relevant anatomic location upon transplantation back into the host. Given the dividing nature of the target cell population, *ex vivo* approaches can employ retroviral and adeno-associated viral vectors.

III. HSV vectors

HSV is a commonly acquired, naturally neurotropic virus that establishes a life-long, benign association with the human host. The virus replicates within epithelial cells of the cornea or orofacial tissue (Cook and Stevens, 1973; Stevens, 1989), invades local peripheral nerve endings and ascends to the associated sensory ganglion by retrograde axonal transport (**Fig. 1**).

HSV is capable of maintaining multiple copies of viral genomes as quiescent episomes within post-mitotic sensory neurons of the peripheral nervous system (Efstathiou et al., 1986; Mellerick and Fraser, 1987; Rock and Fraser, 1985). The HSV genome contains 84 known open reading frames (Roizman and Sears, 1996), approximately half are considered non-essential since they are complemented by host proteins or provide accessory functions that influence viral replication and spread *in vivo*. These can be deleted without affecting the ability of the virus to replicate in culture thus providing the potential to transduce as much as 35 kb of foreign DNA sequence. The current generation of viral vectors exhibit reduced cytotoxicity due to further deletion of viral genes responsible for biochemical and structural alterations in host cell processes that occur in the course of natural infection. These include (i) disaggregation of polyribosomes and degradation of cellular mRNA induced soon after infection by the virion host shut-off protein (vhs) (Kwong et al., 1988; Oroskar and Read, 1989; Read and Frenkel, 1983), (ii) inhibition of RNA splicing by the immediate-early protein ICP27 (Brown et al., 1995; McGregor et al., 1996; Sandri-Goldin and Hibbard, 1996), (iii) fragmentation of host chromosomes, and (iv) destruction of sub-cellular compartments late in infection (Johnson et al., 1992, 1994). Lastly, multiply deleted viral mutants that are incapable of replicating in neurons or any cells other than their stably transformed complementing cell lines have been developed (Marconi et al., 1996; Samaniego et al., 1995; Wu et al., 1996b). These replication-incompetent viral vectors are incapable of reactivating from latency thus providing a relatively safe, gene transfer vector that has a natural propensity for the nervous system.

IV. Overview of relevant HSV biology

The HSV virion contains the 152-Kb double stranded DNA genome packaged in the shape of a torus within an icosadeltahedral capsid (Furlong et al., 1972). The genome consists of two unique segments (UL and US) each flanked by a set of terminal and internal repeats. Surrounding the capsid is an amorphous mass of proteins

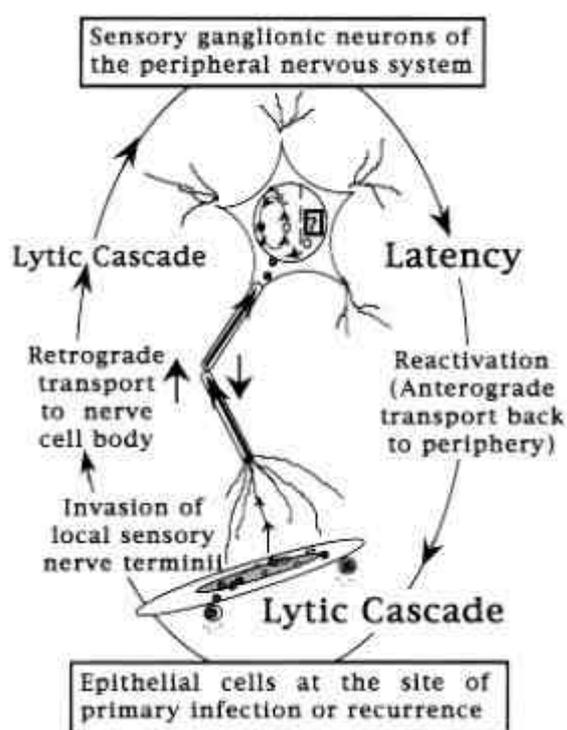


Figure 1. Schematic diagram of the viral life cycle in the host. The stages of the life cycle include: (i) infection of epithelial cells with lytic replication and production of progeny virus particles; (ii) invasion of local sensory nerve endings and ascension to sensory ganglion via retrograde axonal transport; (iii) acute phase of lytic replication in sensory nerve cell body; (iv) establishment of latency and maintenance of viral genomes as quiescent, nucleosome-bound episomes; (v) resumption of lytic gene expression and production of progeny virions upon reactivation that is induced by stimuli such as stress and UV irradiation; (vi) anterograde transport back to the periphery with or without manifestation of symptoms such as cold sores or keratitis.

called the tegument which contains numerous important viral proteins, including the virion host shut-off (vhs) protein that mediates the shut down of host cell protein synthesis (Kwong and Frenkel, 1987; Kwong et al., 1988; Oroskar and Read, 1989; Oroskar and Read, 1987; Read and Frenkel, 1983), and VP16 or α -TIF, a protein involved in transactivating the immediate-early class of viral genes (Batterson and Roizman, 1983; Campbell et al., 1984; Gaffney et al., 1985; Kristie and Roizman, 1987; Mackem and Roizman, 1982; McKnight et al., 1987; O'Hare and Goding, 1988; Post et al., 1981; Preston et al., 1988). Infectious virions possess an envelope acquired by budding through the nuclear membrane. The envelope contains at least ten virus-encoded glycoproteins integrated into the bilayer lipid envelope which are instrumental in the attachment, penetration and cell-to-cell spread of HSV in a variety of different cell types (Spear, 1993a; Spear, 1993b; Steven and Spear, 1997).

A notable feature of the viral lytic cycle is the temporally and sequentially coordinated cascade of viral gene expression (Honess and Roizman, 1974): α -transinducing factor (α -TIF), a component of the virus tegument, induces expression of the first kinetic class of viral genes called the immediate-early (IE or α) genes (Batterson and Roizman, 1983; Campbell et al., 1984; Gaffney et al., 1985; Kristie and Roizman, 1987; Mackem and Roizman, 1982; McKnight et al., 1987; O'Hare and Goding, 1988a; Post et al., 1981; Preston et al., 1988). Four of the five β genes (ICP0, ICP4, ICP22 and ICP27) are involved in transcriptional and post-transcriptional regulation of the next kinetic class of viral genes designated as early or β genes (DeLuca et al., 1985; Dixon and Schaffer, 1980; Preston, 1979b; Preston, 1979a; Rice et al., 1994; Sacks et al., 1985; Sacks and Schaffer, 1987; Sandri-Goldin and Hibbard, 1996; Stow and Stow, 1986; Watson and Clements, 1980). The γ genes provide enzymes required for nucleotide metabolism and viral DNA replication. The last kinetic class of viral genes to be expressed, the δ genes, provide components of the viral icosadeltahedral capsid, teguments and envelope glycoproteins.

A similar cascade of viral gene expression occurs within nerve cell bodies in the sensory ganglia. Infectious viral particles can be detected up to 7 days following infection. However, unlike most cell types sensory neurons are not lysed during the virus lytic cycle. Viral lytic gene expression is repressed by an unknown mechanism and viral genomes are maintained as non-replicating, largely quiescent nucleosome-bound episomes within the nucleus of the sensory neuron until induced to reactivate by stimuli such as stress and exposure to UV irradiation. Upon reactivation, viral nucleocapsids are transported back to the periphery where acute replication resumes within epithelial cells. In humans, manifestations

of viral reactivation include characteristic cold sores or herpetic keratitis, depending on the site of recurrence. Thus, the virus oscillates between two states in the host: long periods of dormancy or 'latency', interrupted by occasional acute periods of active viral replication.

V. HSV latency

During latency, a unique set of transcripts is expressed originating from a 10-Kb region located in the internal (IRL) and terminal (TRL) repeats of the viral genome (**Fig. 2**). The predicted 8.3-Kb primary transcript has proven to be difficult to detect by Northern blot analysis and is therefore believed to be highly unstable or in low abundance. 2.0- and 1.5-Kb length RNA transcripts from within that region accumulate in latently infected sensory ganglia and are referred to as the "latency-associated transcripts" (LATs) (Croen et al., 1987; Deatly et al., 1987; Deatly et al., 1988; Gordon et al., 1988; Rock et al., 1987; Spivack and Fraser, 1988; Spivack et al., 1991; Stevens et al., 1987; Wagner et al., 1988). Recent evidence (Zablotony et al., 1997) suggests that the 2.0- and 1.5-Kb LATs are introns derived from the 8.3-Kb minor LAT (Farrell et al., 1991), a result that is consistent with the nuclear localization, lack of polyadenylation, and non-linear nature of the LATs (Devi-Rao et al., 1991; Wu et al., 1996a).

The functional role of the LATs is not known. Deletions in the LAT region do not have deleterious effects on the ability of the virus to replicate or to establish latency (Block et al., 1990; Chen et al., 1995; Deshmane

et al., 1993; Fareed and Spivack, 1994; Hill et al., 1990; Ho and Mocarski, 1989; Javier et al., 1988; Leib et al., 1989; Natarajan et al., 1991; Sedarati et al., 1989; Steiner et al., 1989), but does appear to delay or reduce the ability of the virus to reactivate (Block et al., 1993; Bloom et al., 1996; Devi-Rao et al., 1994; Hill et al., 1990; Leib et al., 1989; Sawtell and Thompson, 1992; Steiner et al., 1989; Trousdale et al., 1991). Because mutations that affect LAT expression have not been shown to prevent the establishment of latency, it should be possible to exploit the LAT promoter-regulatory region to drive latency-specific expression of a therapeutic gene in place of LAT RNA.

VI. Strategies for engineering HSV vectors

HSV gene vectors have been developed which are mutated in nonessential and essential genes that compromise virus replication in some or all cell types respectively and replication defective mutants have been used to package plasmid vectors which are largely devoid of viral sequences (**Fig. 3**). Vectors mutated in particular nonessential accessory functions are able to replicate in dividing cells (e.g. tumor cells) but not in post-mitotic cells (e.g. brain neurons). Mutants deleted for genes

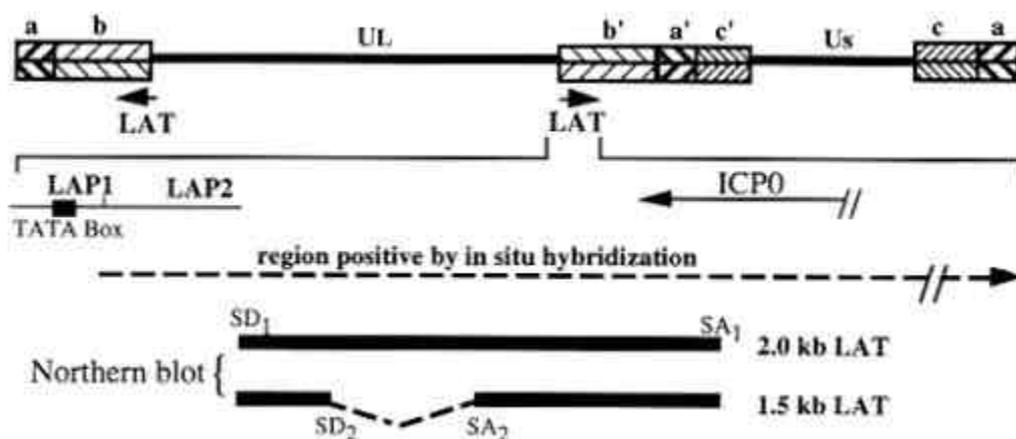
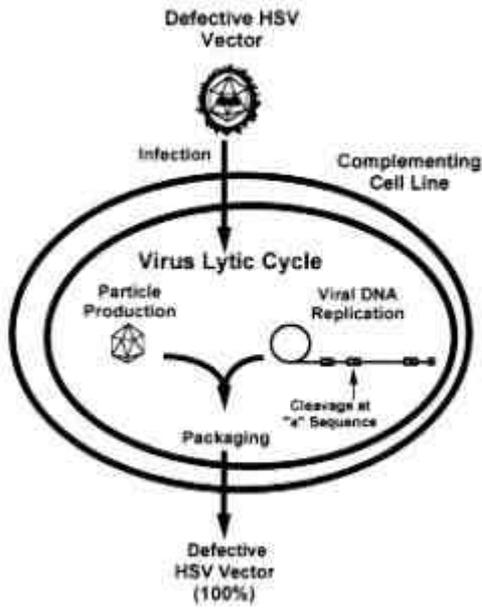
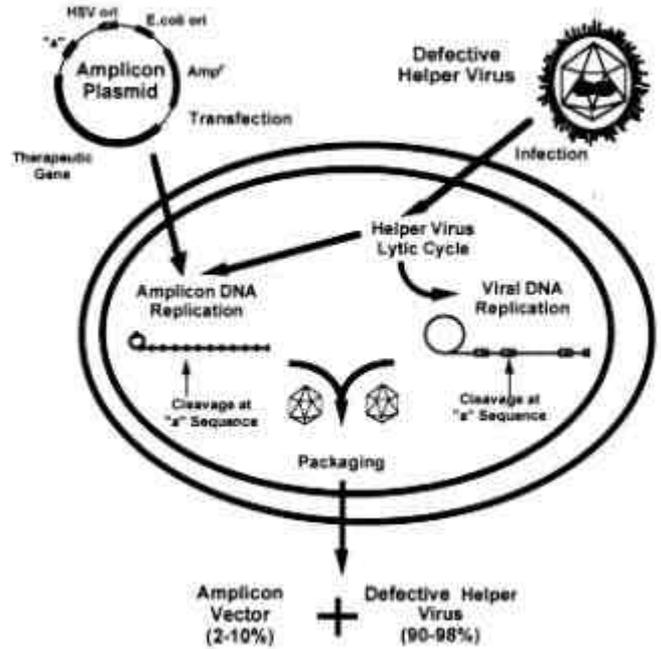


Figure 2. Schematic representation of the LAT loci. Transcription during latency maps to a diploid gene called the LAT locus that maps to the internal and terminal repeats flanking the unique long (UL) segment of the HSV genome. Shown are the location of the two LAT promoter regions, LAP1 and LAP2, relative to the 5' end of the major latency-associated transcripts that accumulate in latently infected ganglia. The dashed line represents the putative 8.7 Kb primary LAT transcript derived from the TATA box-containing LAP1 region. The 2 Kb and 1.5 Kb LATs are co-linear stable introns.

(A) Defective HSV Vector



(B) Amplicon System



(C) Helper Virus-Free Amplicon System

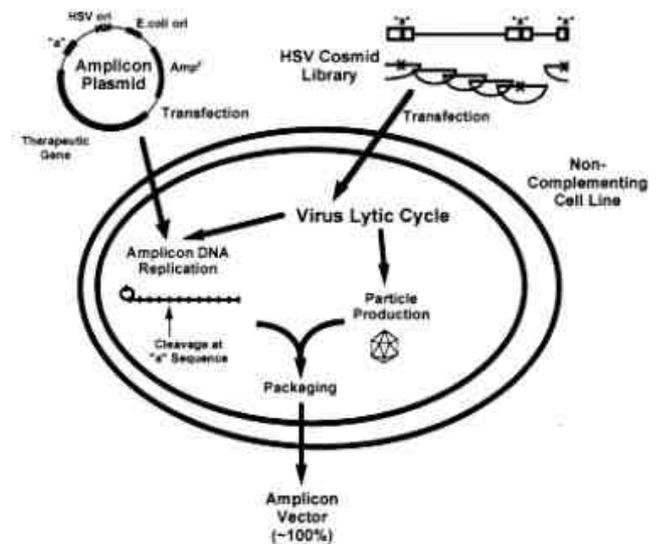


Figure 3. HSV Vector Strategies (A) Production of defective full-length HSV-based vectors is carried out in cell lines that are engineered to provide the deleted essential genes in trans. These vectors are incapable of replicating in neurons because of the missing essential genes. (B) Amplicons are propagated in bacteria (using the bacterial origin of replication), and then transfected into a complementary cell line that is infected with defective “helper” HSV, thus producing particles consisting either of amplicon concatemers (about 150 Kb in length) or defective HSV. (C) Helper virus-free amplicon system doesn’t require either a defective helper virus or a complementing cell line for the amplicon plasmid to be packaged. The amplicon plasmid is transfected into cells along with a five cosmids that contain overlapping fragments that represent the entire HSV genome which encode all the viral proteins necessary to produce infectious particles. In order to insure that only the amplicon plasmid is packaged and not any of the cosmids, the packaging sequence (“a” sequence) was deleted from the cosmid clones.

coding for products involved in DNA synthesis (e.g. thymidine kinase or ribonucleotide reductase) are compromised for growth in brain (Fink et al., 1992; Ramakrishnan et al., 1994) and thus are highly reduced for viral pathogenesis following intracranial virus inoculation. Nevertheless, these mutants can replicate in glioma cells and thus these and other similar mutants have been used as oncolytic vectors for destruction of tumor tissue by the natural cytolytic mechanisms inherent in virus replication (Andreansky et al., 1997; Andreansky et al., 1996; Boviatsis et al., 1994; Chambers et al., 1995; Markert et al., 1993; Martuza et al., 1991; Mineta et al., 1994).

Virus replication and spread within tumors may improve the oncolytic property and also will likely be important for effective delivery of genes which induce anti-tumor immunity or activate anti-cancer drugs locally. There are a number of potential gene knockouts which might allow preferential virus spread in tumor and other tissues many of which have yet to be explored for this purpose. The design of these vectors will require selection of gene deletions which provide the most effective virus spread without compromising vector safety.

Such mutant viruses may also prove useful for gene delivery to sensory neurons since inoculation of skin for example will result in amplification of the vector for more efficient virus delivery to peripheral neurons by virus uptake at axon terminals. Here the virus will establish latency by its inherent mechanisms and transgenes could be expressed using the natural latency promoter system of the virus (described below).

Replication defective virus mutants will be the preferred gene transfer vehicles for applications involving transgene delivery to brain or other tissues where long term expression is required. The safest and most efficient mutant would be deleted for the viral immediate early genes that are required for activation of early and late viral functions. Removal of these genes will substantially reduce viral cytotoxicity and prevent viral antigen production, a problem in the immune competent host where immunologic memory will activate effector T cells that could readily eliminate vector containing cells. There are five IE genes most of which have been shown to be cytotoxic to cells (Johnson et al., 1992, 1994) and mutants deleted for these genes are capable of transducing cells without causing cell death at least at multiplicities of infection of 10 or less (Kriskey et al., 1997; Marconi et al., 1996; Samaniego et al., 1995; Wu et al., 1996b). These vectors will require the use of promoter systems which are active in a quiescent viral genome and may require the use of cellular promoters which are active in particular tissues. Thus far little research has been carried out along these lines since such highly defective mutant viruses have only recently become available.

Plasmid or amplicon vectors have been exploited by a number of laboratories for gene transfer (Battleman et al., 1993; Casaccia-Bonnel et al., 1993; During et al., 1994; Geller and Breakefield, 1988; Geller et al., 1990b; Geschwind et al., 1994; Ho et al., 1993). Amplicon vectors consist of a transgene expression cassette, an HSV origin and packaging recognition sequences. The plasmid is transfected into cells followed by infection with a defective helper virus. Progeny consist of amplicon vector packaged as a concatemer and helper virus particles. Recently this system has been improved by using a cosmid library spanning the entire HSV genome that can not be packaged because they are devoid of packaging signals (Fraefel et al., 1996). While only amplicon-containing particles are produced, high titer stocks are difficult to prepare since the entire system depends on the efficiency of co-transfection. Ideally a packaging system that does not require transfection would provide the best amplicon system however packaging cell lines will be difficult to engineer since at least 35 viral genes are required for particle production and many of these genes are toxic to cells. Amplicons have also encountered problems in maintenance of transgene expression (During et al., 1994; Fraefel et al., 1996) as have other HSV vectors systems and more research on promoter functions in this context is required.

Because HSV is a large virus having many nonessential genes, it should be possible to incorporate large amounts of foreign sequences into the vector genome. Most of the right-hand end of the viral genome (approximately 40 kb) of DNA is nonessential and the two essential genes in this region have been introduced successfully into the genome of cell lines for propagation of highly defective mutants potentially lacking these sequences. All of these sequences have been removed individually or as a large block of genes (Laquerre et al., 1997; Meignier et al., 1988; Rasty et al., 1997; Weber et al., 1987) and experiments are in progress to remove the entire region for replacement with foreign DNA.

VII. Control of transgene expression in HSV vectors

A variety of promoters have been employed to drive reporter/therapeutic gene expression from first generation HSV gene transfer vectors. Candidate promoters included neuronal-specific promoters such as the neuron-specific enolase promoter, the neurofilament promoter and tyrosine hydroxylase promoter, as well as viral promoters such as the HSV thymidine kinase promoter and the constitutively strong HCMV immediate early promoter. Although high levels of reporter gene expression from the HSV lytic cycle or the HCMV IE promoter were detected in rat brain soon after stereotactic injection of the replication-defective

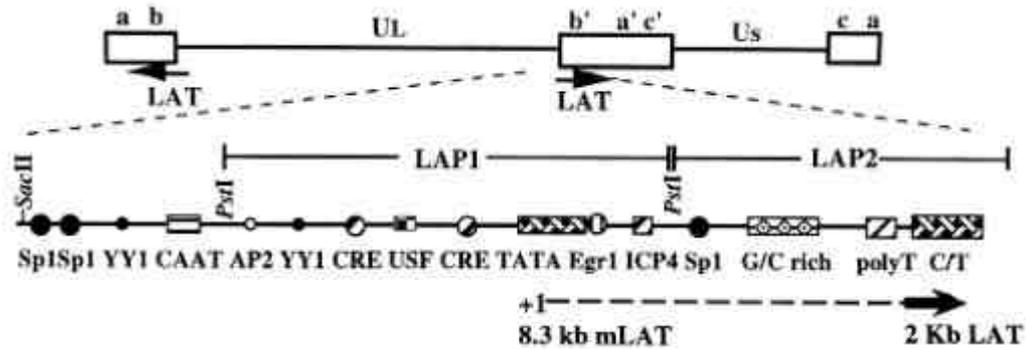


Figure 4. Putative promoter elements within the LAT loci. Schematic representation of the predicted transactivation factor binding sites in the two latency associated promoter regions, LAP1 and LAP2. LAP1 is the TATA box containing promoter located 600 bases upstream of the 5' end of the major LATs within a 203 bp PstI fragment that contains binding sites for several eucaryotic transcription factors including Sp1, USF, CRE, Egr-1 and a member of the POU domain family of transcription factors. LAP2 is a TATA less promoter that resembles several housekeeping gene promoters. LAP2 encompasses several unique sequence motifs such as a GC box, a polyT which binds to a HMG1(Y) and C/T-rich region that binds to Sp1 and a family of factors (PuF, NSEP1, 2F87) that bind to a similar element in the C-myc promoter .

vector, this expression soon waned and could not be detected beyond 7 to 10 days (Fink et al., 1996; Glorioso et al., 1995; Glorioso et al., 1992). At best, expression from the HSV ICP0 promoter and the HCMV IE promoter could be extended out to 4 weeks in the context of a mutant vector background deleted for the IE genes ICP4, ICP27 and ICP22 (Ramakrishnan et al., unpublished), suggesting that one of the immediate early gene products deleted in this vector caused the attenuation of reporter gene expression from previous generation single IE deletion viral vectors.

In our hands the native HSV latency-associated promoter-regulatory region has proven to be the only promoter system that supports sustained gene expression from recombinant HSV-1 vectors in the nervous system (Chen et al., unpublished). The functional contribution of specific cis-acting elements to latency-associated gene expression is currently being assessed in an attempt to build an optimized promoter system using the native LAT promoter-regulatory region as a foundation. We now know that there are two latency active promoters (LAPs), LAP1 and LAP2, upstream of the LAT coding sequence (Batchelor and O'Hare, 1990, 1992; Dobson et al., 1989; 1995; Goins et al., 1994; Nicosia et al., 1993; Wang et al., 1995; Zwaagstra et al., 1989, 1990) (**Fig. 4**). LAP1 contains a TATA box (Ackland-Berglund et al., 1995; Soares et al., 1996; Rader et al., 1993) with upstream control elements such as CAAT (Batchelor and O'Hare, 1992) USF1 (Zwaagstra et al., 1991) CRE (Ackland-Berglund et al., 1995; Kenny et al., 1994; Leib et al., 1991; Rader et al., 1993; Soares et al., 1996) and Sp1,

YY1 and Brn sites. In addition, there is an enhancer region at the start of transcription that plays a role in up-regulating LAP1 basal activity (Soares et al., 1996) and also contains an ICP4 binding site that down-regulates LAT expression (Batchelor et al., 1994; Farrell et al., 1994; Rivera-Gonzalez et al., 1994). We have recently shown that the TATA box, USF1, CRE and the putative Brn site all contribute to LAP1 activity in vivo (Soares et al., 1996, and Soares and Glorioso, unpublished data).

LAP2 resembles housekeeping promoters in that it is TATAless, has a high G + C content, and in transient assays is 5- to 10-fold weaker than LAP1 (Goins et al., 1994). Although LAP2 is also not the predominant promoter during natural HSV latency (Chen et al., 1995), LAP2 can independently drive long term reporter gene expression in the PNS (Goins et al., 1994) (as long as 10 months) and, albeit more weakly, in the CNS (Chen et al., unpublished). Binding of HMG I(Y) protein to a polyT stretch within LAP2 promotes the recruitment of Sp1 and perturbs the local DNA conformation (French et al., 1996).

The ability of LAP1 and LAP2 to drive expression of foreign genes from the virus genome during latency was a natural outcome of the studies characterizing these promoters and the specific cis-elements therein (summarized in **Table 1**). LAP1 was first shown to provide long-term expression of β -globin in mouse PNS in a virus where the β -globin genomic clone was inserted immediately downstream from LAP1 (Dobson et al., 1989), however expression waned dramatically over time (Margolis et al., 1993). A similar virus with the -

interferon (γ -IFN) cDNA at the identical position downstream from LAP1 failed to express γ -IFN during latency (Mester et al., 1995), suggesting that sequences within the β -globin first intron of the genomic clone may have played a role in expression of that particular transgene from the latent viral genome. Another recombinant in which the rat β -glucuronidase cDNA was inserted downstream from LAP1 in a virus that was deleted for part of LAP2 was highly active in expression of the transgene acutely in mouse trigeminal ganglia and brainstem and also expressed β -glucuronidase during latency (Wolfe et al., 1992). However, like the β -globin recombinant, the number of neurons expressing the transgene and the level of expression decreased with time. In other studies LAP1 failed to provide long-term transgene expression either in the native LAT loci (Margolis et al., 1993) or in an ectopic site within the genome (Lokensgard et al., 1994) such as glycoprotein C (gC). Fusion of the Moloney murine leukemia virus (MoMLV) LTR to LAP1 did result in long-term foreign gene expression from the virus vector in neurons of the murine PNS (Lokensgard et al., 1994). Insertion of a MoMLV LTR-lacZ expression cassette 800-bp upstream from the 5' end of the 8.3-Kb LAT in the opposite orientation to LAT led to long-term transgene expression whereas the neurofilament promoter was not active (Carpenter and Stevens, 1996).

Insertion of the transgene immediately downstream of LAP2 in the native LAT loci (Chen et al., 1997; Ho and Mocarski, 1989) or downstream of LAP2 alone in the gC ectopic site (Goins et al., 1994) resulted in long-term activity in both mouse PNS and rat CNS neurons, although the level of transgene expression in brain was reduced compared to that observed in sensory neurons of the PNS. We have also shown that LAP2 is capable of long-term expression of nerve growth factor in trigeminal and dorsal root ganglia neurons in either the tk or gC ectopic loci (Goins et al., unpublished). These results suggest that some element(s) present in the LAP2 region is responsible for mediating expression during latency and that the MoMLV LTR can substitute for that activity. Also, further modification of these sequences will be required to achieve physiologic levels of therapeutic gene expression in brain.

In a recent report, the encephalomyocarditis virus internal ribosome entry site (IRES) was juxtaposed to a reporter gene cassette that was introduced downstream from the LAPs in the native LAT loci, to examine its effect on transgene expression from the LAPs (Lachmann and Efsthathiou, 1997). These recombinants yielded long-term expression of β -galactosidase in murine sensory and motor neurons, although the level and site of expression varied within the population of latently infected cells. Thus, insertion of the IRES may allow for efficient transport of the message to the cytoplasm and thereby increase the level of foreign gene expression.

VIII. Regulated transgene expression in HSV vectors

Our first attempt at engineering a regulatable viral vector created an autoregulatory loop that consisted of a promoter with five tandem copies of the 17-bp Gal4 DNA recognition element to enable transactivation by vector-encoded chimeric Gal4/VP16 protein. This strategy was based on the ability of the Gal4 to transactivate promoters containing this site (Carey et al., 1990; Chasman et al., 1989; Sadowski et al., 1988) despite the repressive presence of nucleosomes (Axelrod et al., 1993; Xu et al., 1993). Completion of the autoregulatory loop achieved enhanced, albeit transient expression of the transgene in the CNS, thus serving as an encouraging proof-of-principle experiment (Oligino et al., 1996). We have since modified this system to achieve an inducible promoter system: the transactivator is a chimeric molecule consisting of the hormone binding domain of the progesterone receptor fused to the previously used transactivation domain of VP16 and DNA binding domain of Gal4 (Vegeto et al., 1992; Wang et al., 1994). In presence of the progesterone analog RU486, the inactive chimeric transactivator assumes a conformation that can bind to and transactivate the Gal4 recognition site-containing promoter driving transgene expression. We have been able to induce high levels of viral vector-derived transgene expression in rat brain upon intravenous administration of the inducing agent RU486 demonstrating the feasibility of the drug-inducible viral vector delivery system (Oligino et al., unpublished).

Table 1. Transgene expression from recombinant HSV vectors in the nervous system. This table summarizes the location and expression profiles of reporter genes inserted into the LAT locus or driven by LAT promoter-regulatory regions in an ectopic genomic locus.

IX. Future directions for HSV vector development.

Herpes simplex virus has many features which make it a promising platform for the creation of vectors for gene transfer to the nervous system. The wild-type virus is capable of naturally establishing long-term persistence in neurons of the brain and peripheral nervous system. Work in our laboratories has focused on modifying the virus to reduce cytotoxicity of the initial infection, and understanding the regulation of gene expression from the

quiescent genome in order to produce either constitutive long-term expression of transgenes in neurons, or regulatable transient expression as required for specific applications. In the peripheral nervous system the virus is well adapted for vector genome persistence and long term gene expression using the native viral constituents and mechanisms for gene expression. Here it remains to be demonstrated that the virus will be effective in treating peripheral nervous system disease in animal model systems. While the virus readily establishes latency in brain, the latency promoter is much less active and will

almost certainly require manipulation to improve promoter function. Possibilities include amplification systems in which the latency promoter is used to express artificially transactivators or the engineering of cis- acting elements into the promoter which are responsive to brain derived transcription factors. Moreover it may be possible to introduce large cellular promoter elements which will be active in the vector genome. Applications involving conditionally replication competent vectors for cancer therapy or spread in vivo will require considerably more research to define a suitable genetic background. These vectors might be improved considerably if virus infection were targeted to particular cell types by modifying the envelope glycoproteins in a manner to eliminate the natural receptor binding ligands with replacement with novel binding ligands that recognize a specific cell type in vivo.

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