

# HSV-1-Based Vectors for Gene Therapy of Neurological Diseases and Brain Tumors: Part I. HSV-1 Structure, Replication and Pathogenesis<sup>1</sup>

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## Abstract

The design of effective gene therapy strategies for brain tumors and other neurological disorders relies on the understanding of genetic and pathophysiological alterations associated with the disease, on the biological characteristics of the target tissue, and on the development of safe vectors and expression systems to achieve efficient, targeted and regulated, therapeutic gene expression. The herpes simplex virus type 1 (HSV-1) virion is one of the most efficient of all current gene transfer vehicles with regard to nuclear gene delivery in central nervous system-derived cells including brain tumors. HSV-1-related research over the past decades has provided excellent insight into the structure and function of this virus, which, in turn, facilitated the design of innovative vector systems. Here, we review aspects of HSV-1 structure, replication and pathogenesis, which are relevant for the engineering of HSV-1 based vectors.

**Keywords:** herpes simplex virus, gene therapy, recombinant HSV-1, amplicon.

## Introduction

In recent years, many neurological diseases have been characterized on a molecular level. The knowledge of the underlying genetic defect and the understanding of related pathophysiological alterations are the first steps toward the development of new treatment strategies based on gene therapy. This form of therapy can be defined as the introduction of exogenous DNA sequences into cells of a target tissue using recombinant DNA and vector technology. The design of effective gene therapy strategies relies on interdisciplinary attempts to: i) define the genetic and pathophysiological alterations associated with the disease; ii) understand the biological characteristics of the target tissue; and iii) develop safe vector and expression systems to achieve efficient, targeted and regulated gene expression. At present, many schemes for gene therapy of both hereditary and acquired diseases have been envisioned, but the logistics of bringing them to humans still needs much basic research [1]. Issues such as efficiency of gene delivery, vector toxicity, stability of transgene and transduced cell, choice of promoter, as well as dose, time and route of vector

application must all be worked out individually for different applications.

In the field of neurology, research in gene therapy and vector technology concentrates on two basic aims, one of which is to achieve stable and non-toxic transduction of neurons and muscle cells for the treatment of neurodegenerative and muscle dystrophic disease, for the alteration of neuronal physiology and conditions with chronic pain, for the control of dystonic movements and stimulation of nerve re-growth. The second aim is the selective and locally toxic transduction of brain tumor cells [2]. For these purposes, a number of different vector systems have been developed, including synthetic vectors, such as molecular conjugates and liposomes, and viral vectors with wide tropism, such as adenovirus (AdV), adeno-associated virus (AAV), retrovirus (RV) and herpes simplex virus type 1 (HSV-1). Synthetic vectors have low toxicity/immunogenicity but poor delivery efficiency, whereas virus vectors can exert some cytotoxicity/immunogenicity but are highly efficient vehicles.

The HSV-1 virion is one of the most efficient of all current gene transfer vehicles with regard to nuclear gene delivery in central nervous system-derived cells, including neurons, neural progenitor cells and gliomas. Many properties of HSV-1 are especially suitable for using this virus as a vector to treat diseases that affect the central nervous system (CNS), such as Parkinson's disease or malignant gliomas. These properties include: i) a high transduction efficiency; ii) a large genome (~152 kb) and a large transgene capacity; iii) the ability of entering a state of latency in neurons; iv) the ability of some mutants to replicate specifically in dividing cells after deleting certain genes required for virus replication in non-dividing cells and, thereby, mediating selective oncolysis of gliomas ("virus therapy"). HSV-1-related research over the past decades has provided excellent

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insight into the structure and function of the virus, which, in turn, facilitated the design of innovative vector systems. However, to bring HSV-1 vector-mediated, targeted and regulatable gene transfer into clinical applications, more technical and logistical issues still need to be addressed.

Here, we review: i) the structure, replication and pathogenesis of HSV-1; and ii) HSV-1-based vector systems with their possible applications in experimental and clinical gene therapy protocols for neurological diseases and brain tumors (Part II).

## Virus Structure

### Genome

HSV-1 is a member of the Herpesviridae, a family of DNA viruses carrying a large, centrally located, linear, double-stranded DNA genome of ~152 kb, which encodes ~80 virus genes [3]. Approximately half of these genes are necessary for virus replication in cell culture. The other half encode accessory functions, which contribute to the virus life cycle in specific tissues or cell types (e.g., postmitotic neurons) of the host (Figure 1) [3–5]. The HSV-1 genome is composed of unique long ( $U_L$ ) and unique short ( $U_S$ ) segments, which are both flanked by inverted repeats ( $R$ ). The repeats of the L component are designated  $ab$  and  $b'a'$ ; those of the S segment are  $a'c'$  and  $ca$ . The HSV-1 genes fall into three categories depending on the kinetics of their transcription, which is tightly regulated in a cascade of three temporal phases: i) immediate early (IE or  $\alpha$ ); ii) early (E or  $\beta$ ); and iii) late (L or  $\gamma$ ) [6].

**Immediate early genes** The immediate early (IE) genes, which encode the infected cell proteins (ICP) 0 ( $IE1=\alpha 0$ ), ICP27 ( $IE2=U_L54$ ), ICP4 ( $IE3=\alpha 4$ ), ICP22 ( $IE4=U_S1$ ), and ICP47 ( $IE5=U_S12$ ), map near the termini of  $U_L$  and  $U_S$  or within the repeats. IE gene products have mostly regulatory functions and initiate expression of the early viral genes. The two open reading frames (ORF) P and ORF O are pre IE genes, map within the repeats, are antisense to the  $\gamma 34.5$  gene, and are expressed under conditions in which ICP4 is not functional.

**Early genes** The early (E) and late (L) genes are distributed throughout both unique sequences,  $U_L$  and  $U_S$ , with only one exception,  $\gamma 34.5$ , which is located in the repeats. Most E gene products are enzymes required for DNA metabolism and signal the onset of viral DNA replication. Seven E genes mapping in the L component (open reading frames  $U_L5$ , 8, 9, 29, 30, 42 and 52) are required for synthesis of viral DNA starting at the origins of DNA replication ( $ori_L$  and  $ori_S$ ). Other early gene products involved in nucleic acid metabolism include uracil DNA glycosylase ( $U_L2$ ), alkaline exonuclease (DNase;  $U_L12$ ), thymidine kinase (TK;  $U_L23$ ), ribonucleotide reductase (RR;  $U_L39-40$ ), deoxyuridine triphosphate nucleotidohydrolase (dUTPase;  $U_L50$ ), and protein kinase ( $U_S3$ ). A unique characteristic of TK is that it phosphorylates purine pentosides and a wide variety of

nucleoside analogs that are not phosphorylated by cellular kinases [7,8]. This substrate specificity of TK is the basis for: i) the effectiveness of various nucleoside analogs in the treatment of HSV-1 infection [9]; ii) TK-mediated pro-drug activation as gene therapy of tumors [10]; and iii) the potential use of radiolabeled nucleoside analogs as “marker substrates” for the non-invasive assessment of TK expression by radionuclide imaging techniques (e.g., positron emission tomography [11–16]). Ribonucleotide reductase reduces ribonucleotides to deoxyribonucleotides, thereby creating a pool of substrates for DNA synthesis [17]. The uracil DNA glycosylase and the alkaline exonuclease encoded by HSV-1 are presumably involved in DNA repair and proofreading [18,19]. The dUTPase hydrolyses dUTP to dUMP, which prevents incorporation of dUTP into DNA and provides a pool of dUMP for conversion to dTMP by thymidylate synthetase.

**Late genes** The late (L) genes encode mainly structural components of the virion [20,21]. Eleven virion proteins (VPs) can be found on the surface of the virion, including glycoproteins (g) L ( $U_L1$ ), gM ( $U_L10$ ), gH ( $U_L22$ ), gB ( $U_L27$ ), gC ( $U_L44$ ), gK ( $U_L53$ ), gG ( $U_S4$ ), gJ ( $U_S5$ ), gD ( $U_S6$ ), gI ( $U_S7$ ), and gE ( $U_S8$ ) [22]. These proteins are glycosylated and play important roles in virus attachment to target cells (gB, gC) [23], cell entry (gB, gD, gG, gH) [24], egress (gG, gH, gK) [25], cell-to-cell spread (gD, gE, gG, gH, gI) [26], and, from the host's point of view, in the induction of neutralizing antibodies (gD, gG, gH/gL) [27,28]. Moreover, gC and gE/gI mediate immune evasion *in vivo*: gC binds complement component C3, thereby inhibiting activation of the complement cascade [29–34]. Glycoproteins E and I form a complex to constitute a high affinity Fc receptor (Fc $\gamma$ R) that binds the Fc domain of human anti-HSV IgG by a process called antibody bipolar bridging and inhibits Fc-mediated immune functions [35–41].

Three or more VPs are intrinsic envelope proteins encoded by  $U_L20$ ,  $U_L24$ , and  $U_L34$ . Seven VPs are found in the capsid in varying numbers, depending on the stage of capsid assembly: VP5 ( $U_L19$ ), VP21+VP22a+VP24 ( $U_L26$ ), VP26 ( $U_L35$ ), VP19C+VP23 ( $U_L38$ ), and VP22 [20,42–44]. Capsid proteins are not only of structural but also of functional importance during encapsidation of viral DNA, e.g., VP22a functions as a scaffolding protein for DNA packaging into capsids [45], and VP19C is thought to be involved in anchoring viral DNA in the capsid [46]. The major capsid protein, VP5, is the structural subunit of both the hexons and pentons comprising the capsomers [47]. The two minor capsid proteins, VP19C and VP23, make up trigonal nodules called triplexes (heterotrimers containing one copy of VP19C and two copies of VP23) found between adjacent capsomers [47,48]. The third minor capsid protein, VP26, is located at the outer tips of the hexons [49,50].

All other VPs are found in the tegument, the space between capsid and envelope. The so-called tegument proteins VP1–2 ( $U_L36$ ), virion host shut off (VHS) protein ( $U_L41$ ), VP11–12 ( $U_L46$ ), VP13–14 ( $U_L47$ ), VP16 ( $\alpha$ -TIF;



not icosahedrally ordered. However, a small portion appears as filamentous structures around the pentons, interacting extensively with the capsid. Their locations and interactions with cellular transport proteins suggest multiple roles in guiding DNA transport into the nucleus [65]. The envelope consists of a lipid membrane, containing glycoprotein spikes on the surface [22], which vary in number and relative amounts, and also including several non-glycosylated viral proteins, lipids, and polyamines.

## HSV-1 Life Cycle

### *Lytic HSV-1 Infection*

The steps of the productive HSV-1 infection include: i) attachment to heparin and related glycosaminoglycans with subsequent binding to specific cell-surface receptors; ii) fusion of the virion envelope with the plasma membrane; iii) transport of the capsid to the nuclear pores with release of the virion DNA into the nucleus; iv) transcription of *IE* and *E* genes; v) viral DNA synthesis; vi) transcription of *L* genes; vii) capsid assembly; viii) DNA packaging; ix) capsid envelopment; and x) virion egress. The individual steps in this cascade are tightly regulated [3], as depicted in Figure 4.

As a first step to establish an infection, low-affinity attachment to the cell surface is mediated by an interaction between envelope glycoproteins C and B and, mainly, cell-surface heparan sulfate proteoglycan, but also dermatan sulfate [23,66–70]. Fusion of the virus envelope with the cell membrane requires at least four viral glycoproteins, gD, gB, and the gH/gL complex [24,71–75]. Two specific cell surface receptors which interact with gD have been identified and designated herpes virus entry mediator (Hve) A and HveC. HveA is a member of the tumor necrosis factor–nerve growth factor receptor superfamily [76–78], and HveC is the poliovirus receptor-related protein 1 [79–82]. HveA activates transcription factors  $\kappa$ B, jun N-terminal kinase, and AP-1, indicating its involvement with signal transduction pathways that activate the immune response [83]. HveA mediates both entry of free virus and entry by cell-to-cell spread [77,84–86]. Moreover, it mediates HSV-1-induced cell fusion [87]. After interaction of viral gD with HveA, gB and the gH/gL complex act individually or in combination to trigger pH-independent fusion of the viral envelope with the host cell membrane [88–92].

The capsid is actively transported along the host cell microtubular cytoskeleton to the nuclear pores [93], where tegument proteins facilitate the release of the virus genome into the nucleus [94] (Figure 4). In the nucleus, the HSV-1 genome is circularized [95], and the virus genes are transcribed in a tightly regulated cascade with three temporal phases. One of the tegument proteins, VP16 ( $\alpha$ -TIF or Vmw65 [96]), binds in the presence of cellular transcription factors to viral DNA at the consensus sequence 5'-GyATGnTAATGARATTCyTTGnGGG-3' [97,98] and induces transcription of *IE* genes [51,96,99] by host RNA polymerase

II. VP16 does not bind directly to the consensus sequence on *IE* promoters, but forms a multiprotein complex with two cellular proteins: the POU domain protein, Oct-1, and a host cell factor (HCF; also called C1, VCAF, or CFF) [63,100–106]. First, VP16 forms a complex with HCF. This association promotes interaction of the complex with Oct-1, which is bound to the TAATGARAT motif. The TAATGARAT motif in *IE* promoters can confer both positive and negative responses to cellular octamer-binding proteins, and the latter results in the absence of *IE* gene expression later in infection and during latency [107]. It should be noted that *IE* promoters also contain consensus sequences for other transcription factors. Like many other viral proteins, VP16 has more than one function. It acts both as a transactivator, which augments the basal expression of *IE* genes, and as an essential structural protein of the virion. Not only VP16, but also proteins involved in cell-cycle control, such as cyclin-dependent kinases (cdk), are important for transcriptional regulation of *IE* and *E* gene expression [108,109].

*Immediate early gene products* HSV-1 expresses five *IE* or  $\alpha$ -genes, *IE*1, 2, 3, 4, 5 (peak rates: 2 to 4 hours post infection, p.i. [6]), which encode the infected cell proteins (ICP) 0, 27, 4, 22, and 47, respectively. Four of these proteins, ICP 0, 27, 4 and 22, regulate the productive cycle of the virus infection by initiating transcription of the *E* genes (peak rates: 5 to 7 hours, p.i.) [6]. ICP47 blocks the presentation of antigenic peptides on the infected cell surface [110–112].

ICP4 and ICP27 are absolutely essential for initiating and controlling the expression of early and also late genes through both transcriptional and posttranscriptional mechanisms [113–119]. ICP4, in concert with basal transcription factors, acts both as transactivator at low-affinity sites and as repressor at high-affinity sites at the transcription initiation signals of its own promoter and those of several other genes [114,120–124]. ICP4 has also been shown to have anti-apoptotic functions [125,126]. ICP27 acts predominantly at the posttranslational level by regulating the processing of viral and cellular mRNAs, thereby contributing to elevated levels of *E* gene products [127–132]. It also contributes to efficient *L* gene expression by acting as a transporter for late viral intronless mRNAs from the nucleus into the cytoplasm [133–135]. The efficiency of nuclear import of ICP27 is modulated by different cellular kinases, such as protein kinase A and C and casein kinase II [136].

ICP22, which shares the C-terminus with the *US1.5* gene product [137], promotes efficient *L* gene expression and regulates the stability and splicing pattern of the *IE*1 mRNA [99,138,139]. It regulates viral gene transcription through modification (phosphorylation) of cellular RNA polymerase II [140]. Furthermore, ICP22 interacts with, and may be stabilized by cell cycle-dependent proteins, such as p78 and p60 [141,142]. The importance of ICP22 for *L* gene expression became apparent from studies showing that concurrent with the onset of viral DNA synthesis, ICP22 and ICP4 aggregate in nuclear structures with nascent viral DNA,

RNA polymerase II, and other proteins, and that this aggregation is essential for late gene expression [143].

ICP0 is a potent transactivator of viral and cellular promoters and is required for efficient viral gene expression and virus replication [144–147]. ICP0 affects different aspects of the host cell metabolism, including cell cycle, proteolytic machinery, transcription and translation [148–151]. ICP0 i) stabilizes cell cycle regulatory proteins (e.g., cyclin D3) to maintain protein synthesis for virus replication [148]; ii) interferes with biochemical mechanisms relevant to both centromeres and ND10 nuclear structures [152,153]; iii) causes active degradation of the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) [154,155]; and iv) binds to a ubiquitin-specific protease, named HAUSP, which contributes to the role of ICP0 in activation of gene expression and stimulation of virus replication [156–158].

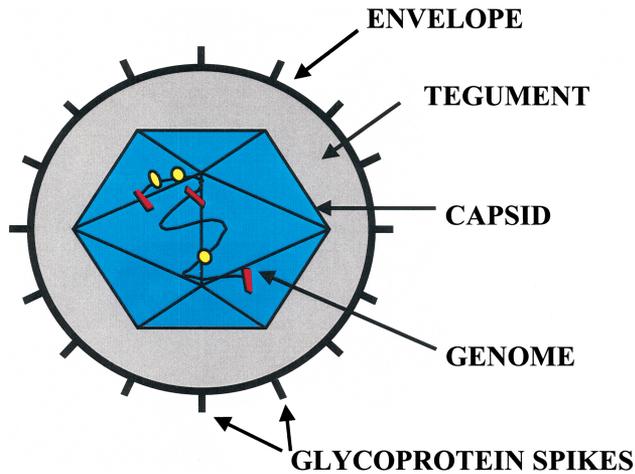
ICP47 binds to the peptide transporter, TAP. This interaction prevents the translocation of peptides into the endoplasmic reticulum (ER) and results in the downregulation of HLA classI/peptide complexes on the surface of infected cells [110–112]. Thus, by expressing ICP47 early in infection, HSV-1 evades detection by CD8+ cytotoxic T lymphocytes (CTL) [159] and prevents CTL-induced apoptosis as mechanisms of immune evasion [160]. In general, viruses have evolved mechanisms to block apoptosis in situations in which endogenously or exogenously induced apoptosis threatens the capacity of the cell to produce the required number and quality of infectious virus progeny [161,162].

*Early gene products* The appearance of the  $\beta$ -polypeptides such as ICP6 (*UL39*; large subunit of viral ribonucleotide reductase, RR), ICP8 (*UL29*; DNA binding protein), ICP36 (*UL23*; thymidine kinase, TK) and DNA polymerase (*UL30*) signals the onset of viral DNA synthesis. At this stage, cellular chromatin becomes degraded. DNA synthesis starts as early as 3 hours p.i. and continues for at least 12 hours. The bulk of the viral DNA is made relatively late in infection [54]. At least seven viral gene products are essential for viral DNA synthesis: i) the products of the *UL5*, *UL8*, and *UL52* genes form a heterotrimeric helicase/primase complex [163]; ii) the products of the *UL30* and *UL42* genes form a heterodimer with processive DNA polymerase activity [164]; iii) the product of the *UL29* gene is a single-stranded DNA binding protein [165]; iv) and the product of the *UL9* gene is an origin binding protein which possesses limited helicase activity [166]. Specifically, the *UL9* gene product interacts with DNA polymerase accessory proteins to provide a means for the ordered assembly of HSV-1 DNA replication proteins at origins of DNA replication, thereby forming a functional “replisome” for the initiation of viral DNA synthesis [53,167]. DNA replication takes place by a rolling-circle, or similar, mechanism [53,168,169], yielding long, head-to-tail linked concatemers of unit-length genomes. Recombination events play an important role in the generation of replication intermediates [170]. The newly synthesized DNA is not

composed merely of linear concatemers, but also contains Y- and X-shaped branches [171].

*Late gene products* In parallel, a third round of transcription results in the production of the  $\gamma$ -proteins which are important for encapsidation of viral DNA and envelopment. The concatemeric products of HSV-1 genome replication are cleaved into unit-length genomes at the DNA cleavage/packaging signals (*pac*) after filling the preformed capsids [172–178]. DNA packaging requires products of the *UL6*, *UL12*, *UL15*, *UL18*, *UL19*, *UL25*, *UL28*, *UL32*, *UL33* and *UL36* genes [179–192]. In cells infected with viral mutants lacking functional *UL6*, *UL15*, *UL28*, *UL32*, or *UL33* genes, unit-length genomes are not cleaved from concatemeric viral DNA [179,182,187,188,191,192]. The *UL17* gene, which is located within the intron of the *UL15* gene, has been demonstrated to encode a tegument protein and was the first tegument-associated protein shown to be required for cleavage and packaging of viral DNA [188]. The *UL25* gene product is not required for DNA cleavage, but is needed for stable retention of DNA in capsids [186]. At 6 to 8 hours p.i., the major capsid protein, VP5, and at least some *UL32* gene products, co-localize in the DNA replication compartment [193–195]. The *UL32* gene product has been shown to guide pre-assembled capsids to the sites of DNA packaging [185]. At these early times, cleavage and packaging seem to occur within replication compartments. Later in infection, VP5 and some tegument proteins accumulate in intranuclear regions separate from the DNA replication compartments, the so-called assemblons [196]. During capsid formation, VP5 and the scaffolding protein, pre-VP22a, condense [197,198] and interact with preformed VP19C–VP23<sub>2</sub> heterotrimers to form procapsids [48,173,176] which mature to capsids.

Mature capsids bud through the nuclear membrane in areas where tegument proteins and glycoproteins have accumulated, thereby acquiring an envelope (Figure 4). The mode of virus egress is not entirely clear. Several models have been proposed, most of which suggest that capsids acquire the envelope at the inner nuclear membrane. Some models suggest that enveloped virions are transported from the nuclear membrane via the ER and Golgi apparatus to the surface without exchanging the envelope [199,200]. However, other models claim that capsids lose their initial envelope by fusion with the outer nuclear envelope (de-envelopment) and acquire a new envelope by budding into the Golgi apparatus (re-envelopment) [201–203]. The ability of HSV-1 gD to interact with mannose-6-phosphate receptors (MPRs) suggests that the intracellular traffic of gD-containing virions might be influenced by the ability of MPRs to direct proteins to endosomes. A vectorial transport of virions to the endosomal network, however, might direct egress to defined domains of the cell surface or promote re-envelopment of capsids within an endosomal compartment [204]. Whatever the mechanism of herpesvirus egress is, mature virions seem to be transported via the ER, the Golgi and trans-Golgi apparatus as well as endosomes into the extracellular space [204,205]. During transit through the



**Figure 2.** Structure of the HSV-1 virion. The HSV-1 virion has a diameter of  $\sim 120$ – $300$  nm and consists of an envelope, the tegument, the capsid, and a core containing the virus genome. The capsid has a diameter of  $\sim 100$  nm and is surrounded by tightly adhering tegument proteins. The envelope consists of a lipid membrane, containing glycoprotein spikes on the surface, which vary in number and relative amounts.

Golgi apparatus, envelope glycoproteins are modified by glycosidases and mannosidases [206]. It should be noted, that the HSV-1 gE/gI complex not only constitutes a high affinity Fc receptor responsible for immune evasion, but also facilitates cell-to-cell spread within the mucosal site of primary infection and infection of sensory nerve terminals by interacting with components of cell junctions, such as  $\beta$ -catenin [26,207,208]. By spreading rapidly from cell-to-cell through a space that is isolated by tight junctions, HSV-1 races against the mounting immune response. This form of direct cell-to-cell spread is the primary mode of virus transmission and an important parameter of HSV-1 pathogenesis.

The entire life cycle of HSV-1 takes  $\sim 18$  to 20 hours, during which the infected cell undergoes major structural and biochemical alterations, ultimately resulting in its destruction. Recombinant HSV-1, which encodes capsid proteins such as VP26 (*UL35*) that are fused with the GFP reporter protein, allows monitoring of capsid assembly and virion formation in living cells over time [209]. An interesting, and potentially important finding is, that expression of HSV-1 glycoprotein gD can prevent re-infection of cells, particularly if the virus has been produced from these cells [210].

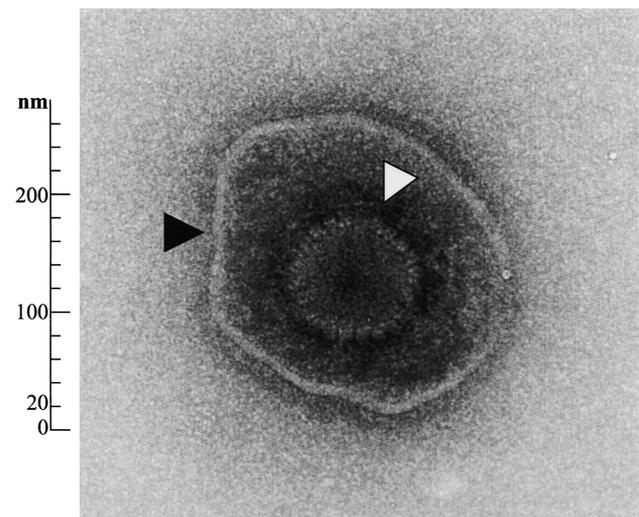
#### Neurovirulence

Any alteration that impairs virus replication reduces virulence and, therefore, all essential genes may be considered “neurovirulence” genes. However, the existence of a specific neurovirulence locus in the long repeat region of the HSV-1 genome is well-documented. This region contains the  $\gamma_{34.5}$  or *RL1* gene which encodes a protein of 263-amino acids, designated ICP34.5 [211,212] (Figure 1). The C-terminal 70-amino acids are highly homologous to the mammalian growth arrest and DNA damage genes, *GADD34* [213–215], and encode two functions. One of these functions enables the replication and spread of the

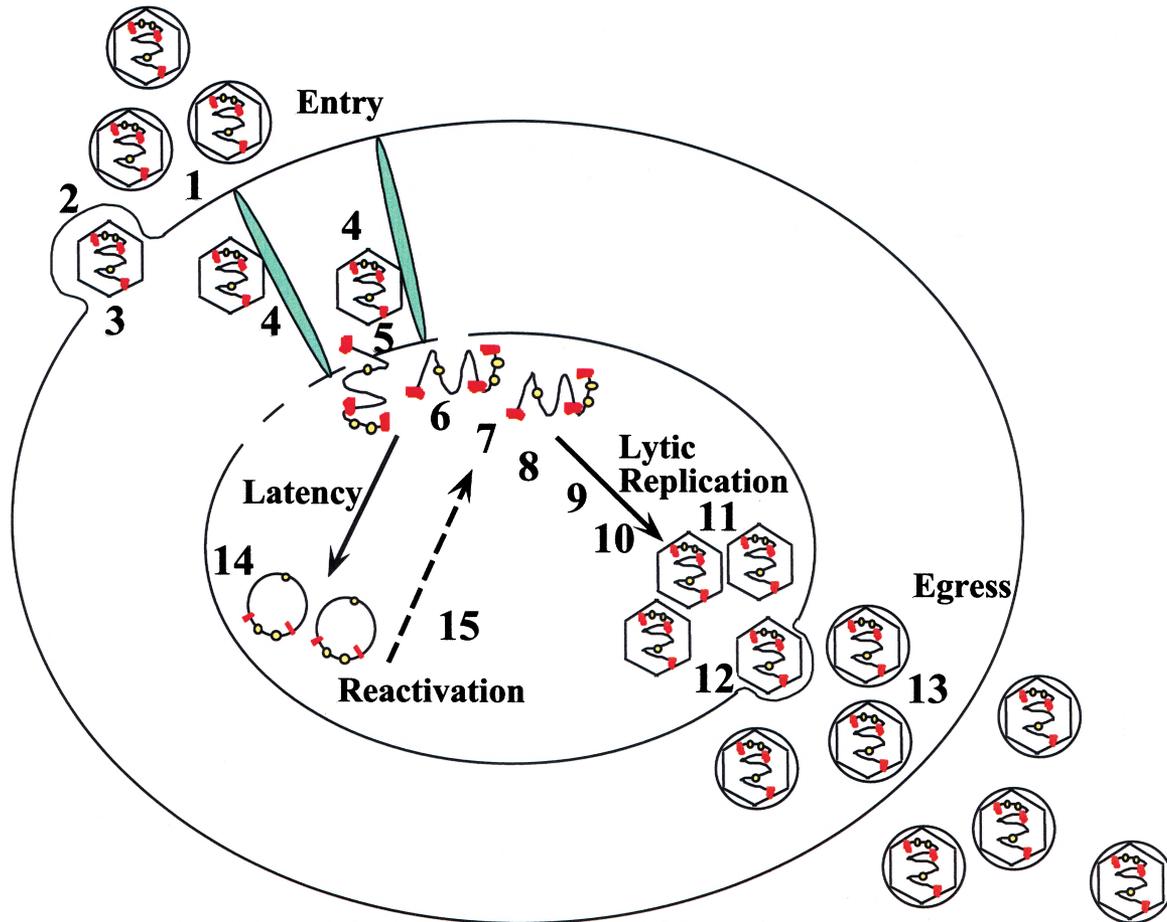
virus in the CNS (“neurovirulence”), especially the maturation and egress from non-dividing cells [216–222]. Null-mutants fail to replicate productively and, hence, do not destroy neurons or cause encephalitis [218,220]. However, they are still capable of establishing and reactivating from latency [223], and retain their wild-type (*wt*) phenotype in permissive cells growing in culture [216,217]. The second function enables the interaction of ICP34.5 with cellular proteins, such as phosphatase  $1\alpha$ , which serves to dephosphorylate the  $\alpha$ -subunit of eukaryotic translation initiation factor 2 (eIF-2 $\alpha$ ). This interaction precludes the premature shut-off of protein synthesis by double-stranded RNA-activated protein kinase, PKR, and prevents apoptosis of infected cells, thus allowing continued virus replication [213–215,224–228]. Other neurovirulence factors include glycoproteins, such as gD, which mediates infection of neural cells, and enzymes involved in DNA metabolism, such as TK, RR, and dUTPase, since mutants in these enzymes cannot replicate in cells in  $G_0$  which have low levels of the complementing cellular enzymes.

#### Latency

The ability of HSV-1 to remain latent in sensory neurons innervating the primarily infected cells for the lifetime of the host is a unique property and is thought to mediate the perpetuation of the virus in the human population. The latent state is characterized by persistence of the virus genome as a non-integrated, concatemeric or circular molecule in the nucleus. During latency, transcription is limited to the latency-associated transcripts (LATs) and no viral proteins are synthesized [229–232]. Latently infected neurons are not rejected by the host immune response and appear to function normally. These properties make HSV-1 an interesting candidate vector for gene delivery to cells of the nervous system.



**Figure 3.** Electronmicrograph of an HSV-1 virion. Envelope ( $\blacktriangle$ ) and capsid ( $\triangle$ ) are clearly delineated (a kind gift from Drs. Elisabeth Schraner and Peter Wild, University of Zürich).



**Figure 4.** Lytic and latent HSV-1 infection. The lytic HSV-1 life cycle takes ~18 hours and the steps include: 1) attachment to heparan sulfate and cell-surface receptors; 2) fusion of the virion envelope with the plasma membrane; 3) release of the capsid into the cytoplasm and 4) active transport along microtubules to the nuclear pores with 5) release of the virion DNA into the nucleus; 6)  $\alpha$ -TIF mediated induction of transcription of IE genes; 7) transcription of IE and E genes; 8) viral DNA synthesis; 9) transcription of L genes; 10) capsid assembly; 11) DNA packaging into preformed capsids; 12) capsid envelopment; and 13) virion egress. In sensory neurons, HSV-1 may enter a state of latency 14), which is characterized by the persistence of the HSV-1 genome as a concatemeric or circular molecule bound by nucleosomes that does not express viral genes other than the latency associated transcripts. Host cell, viral and external factors play a role in establishment and reactivation 15) of HSV-1 from latency.

The exact mechanism of HSV-1 latency and reactivation is not known, and the following description can only serve as a model, which still has to be elucidated in detail [3]. Virus replication at the site of the primary HSV-1 infection supports entry of the virus into the nerve endings. From there, the virus capsids are transported retrogradely along microtubules to the sensory ganglia (migration rate ~1 cm/hour [233]), where latency is established. There is a direct correlation between the amount of input virus and the number of neurons that become latently infected [234]. As mentioned above, the HSV-1 genome is transcriptionally silent during latency except for the LATs [235–241]. LAT has been suggested to act in an antisense manner to block ICP0 activity because the two genes overlap and are transcribed in opposite orientations [240]. However, the exact functions of the LATs during latency remain elusive. LAT expression is not required for establishment or maintenance of latency [242,243], but it serves to increase the number of neurons in which latency is established [244], and it is involved in the efficiency of reactivation [243,245–249].

The LAT region consists of several genetic elements [250]. Two promoter elements responsible for expression of LATs have been identified, LAP1 and LAP2 [251–255]. The LAP1 promoter, which includes a TATA-box, and USF, CRE, AP1, and POU binding domains, is primarily responsible for LAT expression during latency [256]. The combined deletion of USF, CRE, and TATA-box completely abolishes LAT transcription in the brain, identifying these elements as essential for the neuronal specificity of LAP1 during latency. In cell culture, LAP2 is primarily active following viral DNA synthesis and, hence, responsible for LAT expression during lytic infection [257]. The LAP2 promoter is located between LAP1 and the LAT intron and does not contain a TATA-box, but has homology to mammalian housekeeping gene promoters [254,255,257].

Both host cell- and viral factors seem to play a role in the establishment and maintenance of latency in that: i) neurons destined to harbor latent virus may not express Oct1 [258]; ii) ICP0 seems to play a role in establishment of latency [259] and reactivation [259,260]; iii) a NGF/FGF-inducible cellular

factor may be responsible for the initiation of viral gene expression during reactivation when no ICP0 is present [261]; iv) deletion of the  $\gamma 34.5$  gene markedly impairs the ability of the virus to establish latency [262]; v) mutations that result in reduced efficiency of virus replication have a negative effect on both the establishment of latency and the ability to reactivate [144,146,263,264]; and vi) the number of HSV-1 genome copies within individual, latently infected neurons is regulated by viral genetic factors [265].

The earliest molecular events in neurons that trigger reactivation of HSV-1 remain unclear, but may include altered expression of cellular factors such as the induction of transcriptional activators and downregulation of repressors. However, a temporal link between virus reactivation and induction of cellular *IE* genes encoding *c-fos*, *c-jun*, *c-myc*, Oct-1, TIS7, IFN, and IRF-1 has not yet been established [266–268]. The probability of virus reactivation increases with the number of latently infected neurons in the ganglia [269]. Well-known reactivation factors, such as physical and emotional stress, peripheral tissue and axonal damage, fever, UV light, hormonal imbalance, malignancy or immune suppression, may reactivate virus replication, followed by concurrent axonal transport of the virus progeny, usually to the site of the primary infection. Repeated reactivation events do not appear to kill the neuron, and thus, the extent of virus replication must be limited.

### HSV-1 Pathogenesis

HSV-1 is transmitted from infected humans to susceptible individuals during close personal contact [270,271]. HSV-1 replicates initially in surface epithelial or mucosal cells, with subsequent spreading to cells of the nervous system via infection of nerve terminals that innervate the site of primary infection. *Primary* HSV-1 infection is usually established before the age of 5 years after an incubation period of several days (mean: 4 days). Great variability exists in the symptoms which may be subclinical or include variable combinations of fever and malaise, sore throat, gingivo-stomatitis and lymphadenopathy. Virus replication at the site of infection causes a localized vesicular or ulcerative lesion leading to edema, usually in the oropharyngeal mucosa. However, almost any organ can be infected with this virus. HSV-1 keratoconjunctivitis is a major cause of corneal blindness, and, even with appropriate antiviral therapy, healing of corneal ulcers may take several weeks. HSV-1 skin infections (eczema herpeticum) usually occur in patients with atopic dermatitis. The lesions can be either isolated or disseminated and may trigger an erythema multiforme. Especially in the immunocompromised host, progressive disease may cause virus dissemination with infection of the skin, the respiratory tract, the esophagus, and the gastrointestinal tract. The clinical manifestations of neonatal HSV-1 infection are summarized elsewhere [271].

From the site of primary virus replication, nucleocapsids are transported via sensory neurons to the dorsal root ganglia (e.g., trigeminal ganglion Gasser), where the virus enters either a lytic pathway with subsequent production of

progeny virus particles or a latent state [233,272,273] (Figure 4). Certain stimuli can cause reactivation from latency with local virus replication within the ganglion, concurrent axonal transport of virions to the sites of mucosal membranes or the skin and subsequent *recurrent infection* (e.g., herpes labialis or keratoconjunctivitis). Clinical distinction should be drawn between intraoral gingival lesions, indicative of primary infection, and lip lesions, indicative of recurrent infection. More than a third of the human population have recurrent HSV-1 infections, which may even occur in the absence of clinical symptoms. Although usually the host–virus interaction leads to latency and recurrent infections within the periphery, rarely virions are transported into the CNS, and may cause life-threatening CNS infections, most commonly as hemorrhagic encephalitis of the temporal lobe. Symptoms of HSV-1 encephalitis include fever, altered consciousness, behavioral abnormalities and localized neurologic findings and seizures. Other manifestations include radiculitis, myelitis or meningitis similar to complications mediated by varicella zoster virus [274]. Thus, key elements in HSV-1 pathogenesis are the interaction of the virus with the nervous system and the immune system.

On average, virus shedding in mouth and stool occurs for 7 to 10 days. Neutralizing antibodies appear between 4 to 7 days after clinical onset and peak  $\sim 2$  weeks later. In addition, macrophages, natural killer cells, specific sub-populations of T-cells (CD4+, CD8+) and a variety of cytokines (e.g., type 1: IL-2, IFN- $\gamma$ , IL-12; type 2: IL-4, IL-5, IL-10, IL-13) take part in the complex immune response against HSV-1 infection [275,276]. Humoral immunity does not prevent exogenous reinfection or recurrences, but does limit spread of the virus in the host.

The diagnosis of HSV-1 is made by virus isolation or detection of viral DNA by PCR from vesicles, nasopharynx, conjunctivae, cerebrospinal fluid, stool and urine, and by serology of rising specific antibodies. In the event of encephalitis, magnetic resonance imaging and electroencephalography reveal the structural and functional consequences of the destructive inflammation. Herpes encephalitis is treated with acyclovir, which can be effective if administration begins early (within 48 hours) in the course of CNS infection.

### Conclusion

The mounting knowledge of HSV-1 structure and physiology has facilitated the development of tools for the rapid diagnosis and effective treatment of HSV-1 infections as well as the construction of HSV-1-derived vectors. There are two principle types of HSV-1-based vector systems: recombinant HSV-1 vectors and HSV-1 amplicon vectors. The engineering and possible applications of these vectors are discussed in detail in Part II of this review.

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