

Review

Genus Simplex Virus: Classification, Morphology and Genome Organization

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Abstract

Human herpesviruses (HHVs) are ubiquitous infectious agents of anthroponotic viral infections worldwide. They are responsible for the occurrences of recurrent diseases with a wide range of clinical manifestations. The purpose of this overview is to summarise our current knowledge of the genus *Simplexvirus* by examining its classification, morphology, and genome organization.

Keywords: genus Simplexvirus, Herpes simplex virus-1 (HSV-1), Herpes simplex virus-2 (HSV-2), classification, taxonomy, genome organization

Резюме

Човешките херпесвируси (HHVs) са повсеместни инфекциозни агенти на антропонозни вирусни инфекции по целия свят. Те са отговорни за появата на рецидивиращи заболявания с широк спектър от клинични прояви. Целта на този преглед е да обобщи настоящите ни познания за рода *Simplexvirus* чрез изследване на неговата класификация, морфология и организация на генома.

Classification and taxonomy of genus *Simplexvirus*

Historically, herpesvirus taxonomy has been addressed since 1971 by the International Committee on Taxonomy of Viruses (ICTV) (Davison, 2007). According to the latest ICTV taxonomic edition of October 2020, human herpes viruses (HHVs) belong to the family *Herpesviridae*, which in turn consists of three subfamilies, accommodating 17 genera and 115 species. Based on their biological properties as well as growth requirements and tissue tropism, herpes viruses can be further subdivided into three subfamilies (Davison, 2007). The subfamily *Alphaherpesvirinae* includes five genera: *Iltovirus*, *Mardivirus*, *Scutavirus*, *Simplexvirus*, and *Varicellovirus*. Characteristically, members of this taxon persist in the sensory ganglia as a latent infection which may reactivate under stressful conditions or with changes in the human organism undoubtedly related to the immune system. The body's weakened natural defense system "authorizes" the start of replication and the migration of neurotropic herpes viruses along the myelin sheath

of neurons, thus causing the formation of mucocutaneous lesions. Etiological agents of anthroponous infections are members of the last two genera. Two infectious agents of the same name, *Human herpesvirus-1/Herpes simplex virus-1* (HSV-1) and *Human herpesvirus-2/Herpes simplex virus-2* (HSV-2) belong to the genus *Simplexvirus*. The causative agent of varicella (chickenpox), *Human herpesvirus 3/Varicella-Zoster virus* (VZV), is a member of genus *Varicellovirus*.

The *Betaherpesvirinae* subfamily also consists of five genera: *Cytomegalovirus*, *Roseolovirus*, *Muromegalovirus*, *Proboscivirus* and *Quwivirus*. Members of this taxon primarily cause asymptomatic infections in immunocompetent individuals or latent infections in several cell types, including various types of leukocytes (Spear and Longnecker, 2003). The first two genera are related to humans. *Human herpes virus-5/Cytomegalovirus* (CMV) is recognized as the most common cause of congenital viral infections in humans and main factor for morbidity and mortality in immunocompromised hosts (Ross *et al.*, 2011). This virus is the most import-

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ant member of the genus *Cytomegalovirus*. *Human herpes virus-6/Herpes lymphotropic virus* (HHV-6) and *Human herpes virus-7* (HHV-7), which belong to the genus *Roseolovirus*, are ubiquitous and associated with *roseola infantum*, *pityriasis rosea*, *lichen planus*, hypersensitivity reactions, graft-versus-host disease (GvHD), and numerous other skin manifestations (Wolz *et al.*, 2012).

Gammaherpesvirinae is a subfamily whose members are known as lymphotropic herpesviruses. These infectious agents cause lifelong infections and replicate mainly in T- and B-lymphocytes, with strong oncogenicity under certain conditions, inducing mutations in certain genes, causing Hodgkin's lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, gastric carcinoma, and nasopharyngeal carcinoma. The subfamily consists of seven genera: *Bosavirus*, *Macavirus*, *Manticavirus*, *Patagivirus*, *Percavirus*, *Rhadinovirus* and *Lymphocryptovirus*. The last two are of medical importance. *Human herpes virus-4/Epstein-Barr virus* (EBV) can cause a variety of diseases and is transmitted mainly through the saliva, which contains infected epithelial cells. Nearly 95% of adults worldwide are infected (Hoover and Higginbotham, 2021). EBV infection is often asymptomatic or has diverse pathological manifestations: from infectious mononucleosis to severe neoplastic diseases of epithelial and lymphocytic origin (Stanfield and Luftig, 2017). Human herpesvirus-4 belongs to the genus *Rhadinovirus*. *Human herpes virus-8/Kaposi's sarcoma related virus* (HHV-8/ KSHV) is a member of the genus *Lymphocryptovirus*. Unlike the other human herpes viruses (HSV, EBV, VZV, CMV, HHV-6, and HHV-7), it is not widely distributed in the general population and possesses many unique proteins. HHV-8 has been identified in all subtypes of Kaposi's sarcoma (KS), multicentric Castleman disease, and a rare form of B-cell lymphoma, primary effusion lymphoma (PEL) (Ablashi *et al.*, 2002).

In terms of structural relationships and taxonomic rank of genus *Simplexvirus* in the evolutionary developed biological systems, members of this taxon meet common morphological, biological and genomic criteria. Affinity to cell types during the establishment of latent infection is the leading factor uniting Herpes simplex virus-1 (HSV-1) and Herpes simplex virus-2 (HSV-2) into a common genus. While other human herpes viruses exhibit cellular tropism towards cells of the myeloid lineage (CMV, HHV-6, HHV-7) and lymphocytes (EBV, KSHV), herpes simplex viruses attack neurons. This biolog-

ical feature determines the viral pathophysiology often associated with the severe infectious disease herpes simplex encephalitis (HSE).

Morphology of the genus *Simplexvirus*

Members of the genus *Simplexvirus* show a strong morphological uniformity with the other members of the family *Herpesviridae*. Virions have complex and characteristic structures consisting of both symmetric and asymmetric components (King, 2011). The spherical virion has a unique four-layered structure: a core enclosed in an icosahedral nucleocapsid made up of capsomeres. The capsid is surrounded by an amorphous protein coat called the tegument, itself encased in a glycoprotein-bearing lipid bilayer envelope (Whitley, 1996). The virion is approximately 125 nm in diameter. The viral genome is packaged as a single linear double-stranded DNA molecule (dsDNA) in the core (ICTV) (King, 2011). Members of the genus possess one of the largest infectious genomes, with multiple open reading frames (ORFs). The HSV-1 genome is approximately 152 kb in size and is known to encode at least 80 open reading frames (ORFs), many of which have been well studied (Whisnant *et al.*, 2020). At least 84 unique open reading frames (ORFs) have been recognized in HSV-2, along with several RNA transcripts that have not been shown to also encode proteins (Minaya *et al.*, 2017). Human simplex viruses have a G + C content of approximately 68% (Whitley, 1996).

Morphologically, the tegument is a structure unique to herpes viruses, occupying the space between the nucleocapsid and the envelope, and containing numerous virus encoded proteins called tegument proteins (Guo *et al.*, 2010; Oda *et al.*, 2016). From a functional point of view, the location of the tegument in mature virions indicates that this morphological structure links the nucleocapsid to the bilayer glycoprotein lipid envelope, maintaining the structural integrity of infectious particles and interacting directly with capsid proteins, other tegument proteins, and the cytoplasmic domains of envelope glycoproteins and/or the membrane-bound proteins of the tegument (Oda *et al.*, 2016). It is for this reason that the amorphous shell is subdivided into an "inner" layer, which is tightly bound to the capsid, and an "outer" layer, bound to the bilayered envelope (Scrima *et al.*, 2015). Depending on the type of herpes virus, the thickness of this structure ranges from 20 to 40 nm, and it may be uniformly or asymmetrically arranged around the capsid (Newcomb and Brown, 2009). The tegument structure has been described as fibrous or granular,

and changing as the virus particle matures. Under a microscope, the tegument is seen as a relatively low-density morphological region covering an area of 60–100.0 nm in radius (Davison, 2007). Studies on herpes simplex virus type 1 (HSV-1) have shown that the tegument structure in the cell-associated form is altered compared to the extracellular virion (Newcomb and Brown, 2010). It is known that the tegument plays a key role not only in the structural aspects of viral morphology, but is also involved in many essential biological processes, such as the viral life cycle, the translocation of the nucleocapsid to the cell nucleus, as well as the assembly and egress of the infectious progeny from the host cell (Guo *et al.*, 2010).

The tegument, made up of thousands of densely packed protein molecules (Owen *et al.*, 2015), accounts for 40% of the virion protein mass (Davison, 2007; Newcomb *et al.*, 2012). A proteomic analysis of extracellular HSV-1 virions by mass spectrometry has identified 23 tegument proteins and numerous cellular enzymes, chaperones and structural proteins, some of which probably incorporated in the protein layer (Owen *et al.*, 2015). HSV-1 tegument structural proteins range in size and abundance, with the smallest being approximately 10.5 kDa (UL11), and the largest more than 330 kDa (UL36/VP1-2). The most abundant proteins embedded in the amorphous layer are tegument protein (UL47/VP13-14), tegument protein (UL48/VP16), and tegument protein UL49/VP22, which are present at 600–1300 copies per virion (Owen *et al.*, 2015; Newcomb and Brown, 2009). The other tegument proteins may occur in 100.0 or fewer copies. At the same time, traces of cell-encoded proteins are also present (Newcomb and Brown, 2009). During virion morphogenesis, viral tegument proteins (VPs) facilitate viral envelopment by establishing a protein network found within the enveloped virions. In support of this hypothesis, it has been reported that a number of HSV-1 tegument proteins, nuclear egress protein 1 (UL31/NEC 1), nuclear egress protein 2 (UL34/NEC 2), large tegument protein (UL36/VP1-2), inner tegument protein (UL37), tegument protein (UL14), tegument protein (UL47/VP13-14), tegument protein (UL48/VP16), cytoplasmic envelopment protein 3 (UL11), and cytoplasmic envelopment protein 2 (UL16) are required in the effective viral primary or secondary envelopment (Oda *et al.*, 2016). Tegument proteins are classified as inner or outer, based on their association with the nucleocapsid once it has entered the host cell cytoplasm. The inner tegument protein

(UL36), the inner tegument protein (UL37), and the serine/threonine-protein kinase (US3) remain associated with the capsid after virus entry, while the other (outer proteins) dissociate from the overall structure of the virion (Newcomb *et al.*, 2012).

The icosahedral nucleocapsid is made up of 161 capsomeres (150 hexons and 11 pentons), a portal complex with an axial channel through which double-stranded infectious DNA enters and exits the capsid, 320 triplexes that connect the capsomeres and the portal complex, small capsomere-interacting proteins (SCPs), and capsid vertex-specific complexes (CVSCs) that are rod-shaped with five rods located near each capsid vertex (Kobayashi *et al.*, 2017). In herpes virus capsids, both pentons and hexons are cylindrical in shape (approximate diameter 140 Å, height 160 Å) with a central, axial channel approximately 25 Å in diameter. The penton and hexon subunits have an elongated shape with multiple domains, including upper, middle, lower, and floor domains. The middle domains of the subunits interact with one another as triplexes. The lower domains connect the subunits to each other and form the axial channels. While the upper domains of adjacent hexon subunits interact with one other, the penton subunits are disconnected at their upper domains, resulting in the V-shaped side view of the penton (Davison, 2007). Morphologically, the capsid of all herpes viruses, including herpes simplex viruses (HSV), contains 12 vertices. Eleven are identical and comprise 5 copies of the major structural HSV capsid protein (VP5/UL19), while the 12th vertex comprises 12 copies of the portal protein UL6, forming the portal complex (Yang *et al.*, 2014). In HSV-1, the eleven identical capsid structures, designated pentons, are linked to adjacent hexon proteins by triplexes located on the capsid surface. Hexons in turn are composed of five or six VP5 molecules (monomers). Capsid vertex-specific complexes (CVSCs) comprise a single protein molecule UL17 and one molecule UL25. The triplexes are made of a single VP19C molecule and two VP23 molecules, whereas the portal complex is composed of 12 UL6 molecules. HSV-1 capsomere-interacting proteins (VP26) form a hexameric ring positioned on the outer surface of each hexon (Yang *et al.*, 2014; Kobayashi *et al.*, 2017).

Three capsid forms - A, B, and C - can be differentiated in the host cell nucleus during infection (Tandon *et al.*, 2015; Yuan *et al.*, 2018; Wang *et al.*, 2018). Types A and B result from problems in viral genome packaging. For this reason, they are also described as incomplete structures. Type A cap-

sids characteristically do not contain either genetic information (dvDNA) or scaffold proteins while type B capsids only lack viral genome (Kobayashi *et al.*, 2017). On the other hand, type C capsids are defined as mature capsids containing viral genome but without scaffold proteins (Kobayashi *et al.*, 2017). These capsids are capable of maturing into potentially infectious virions (Conway *et al.*, 2010). All three types of capsids (A, B and C) have angular shapes typical of the *Herpesviridae* family (triangulation number $T = 16$). They are composed primarily of 955 copies of the major capsid protein (UL19/VP5) of 150 kDa, arranged as 150 hexons and 11 pentons (Wang *et al.*, 2018). In addition to the main capsid protein (UL19/VP5), the nucleocapsid is composed of three more virion proteins: capsid triplex subunit 1 (UL38/VP19c), capsid triplex subunit 2 (UL18/VP23), and a small capsid protein (UL35 / VP26) (Sheaffer *et al.*, 2000).

Morphologically, the envelope is one of the most important structures with a fundamental role in the process of virus infectivity. The glycoprotein lipid bilayer is cell-derived as it is derived from internal membranes of the host cell (Wudiri *et al.*, 2017). Herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) are now known to possess at least 12 viral glycoproteins bound to the envelope (gB/UL27, gC/UL44, gD/US6, gE/US8, gG/US4, gH/UL22, gI/US7, gJ/US5, gK/UL53, gL/UL1, gM/UL10 and gN/UL49.5) (Sari *et al.*, 2020; Madavaraju *et al.*, 2021). They also encode several non-glycosylated viral proteins, lipids and polyamines (Whitley and Roizman, 2002). Some of the glycoproteins occur as macromolecular complexes - heterodimers (gH/gL and gE/gI), while most exist as monomers (Madavaraju *et al.*, 2021). Glycoproteins B, D and H/L (gB, gD and gH/gL) are responsible for the entry of the infectious agent into the host cell, while glycoprotein C (gC) facilitates low-pH entry and infection, protecting gB against humoral immune response and its binding to neutralizing antibodies (NAbs) (Sari *et al.*, 2020).

Glycoprotein E (gE) plays a key role in host cell recognition, which is an important step of virus infection. The members of the subfamily *Alphaherpesvirinae* (HSV, VZV and PRV) express a heterodimer of two membrane glycoproteins, gE and gI, whose joint function is responsible for the protein complex-mediated cell-to-cell spread in epithelial and neuronal tissues (Johnson *et al.*, 2001). In neuronal cells, the gE/gI heterodimer facilitates axonal transport of capsids from the soma, bringing them closer to kinesin motor proteins to allow

the anterograde transport. Thus, the heterodimer may use the same mechanism during reactivation of lytic infection by facilitating the spread of HSV infectious particles from the neuronal cell body to epithelial cells (Banerjee *et al.*, 2020). At the same time, the glycosylated gE protein actively interacts with some of the tegument proteins (UL11, UL16 and UL21) (Han *et al.*, 2012). Glycoprotein M (gM) participates in several important stages of viral invasion, namely in virus-induced cell fusion, followed by nucleocapsid release to the cytoplasm, and the assembly and release of infectious progeny (Li *et al.*, 2021). The 12th glycoprotein N (gN) plays a key role in viral morphogenesis - for maturation and modulation of the fusion activity of gM, ensuring the membrane transport of the virus (Kasmi and Lippépes, 2015). Glycoprotein K (gK) interacts with the membrane protein (UL20) to form a functional protein complex. This dependence ensures (gB)virus-induced membrane fusion (Chouljenko *et al.*, 2010), the subsequent exit of the infectious particles from the nucleus, and the formation of the viral envelope (Rider *et al.*, 2019).

Glycoprotein G (gG) of herpes simplex viruses HSV-1 and HSV-2 (gG1 and gG2, respectively) is implicated in the induction of immune response. Because of the cascade of inflammatory responses it initiates, gG has been described as a viral chemokine-binding protein (vCKBP) (Walle *et al.*, 2007). HSV is characterized by exerting strong modulatory pressure on the host immune system. This occurs under the control of viral chemokine-binding proteins (vCKBP) expressed by the infectious agent, which are high-affinity binding to chemoattractant cytokines, inhibiting their immunological functions. The sole exception is glycoprotein G, whose immunogenic functionality enhances chemokine-mediated cell migration and signalling (Martinez-Martin *et al.*, 2015). Studies carried out in 2018 demonstrated that glycosylated protein J (gJ) promotes cell-to-cell spread of HSV-2 virus infection and syncytia formation (Liu *et al.*, 2018). The same study by Liu *et al.* (2018) indicated that gJ knock-out or knockdown impairs the cell-to-cell spread of the virus (from epithelial cells to epithelial cells or to neurons), which in turn leads to reduced generation of infectious virus particles.

Genomic organization

Viral linear dsDNA contributes to the realization of the infectious potential of herpes simplex viruses. The HSV-1 has a genome size of about 152 kb and encodes at least 80 open reading frames (ORFs) (Whisnant *et al.*, 2020), which

in turn encode a multitude of proteins, including transactivator of transcription proteins; non-structural proteins (NSPs) required for DNA replication; RNA-binding proteins, proteases, kinases, and structural proteins (SPs) which constitute the infectious virus particle (Arachchige *et al.*, 2018). Besides these ~84 transcripts, the genomes of herpes simplex viruses (HSVs) also encode numerous long non-coding RNAs (lncRNAs) and 16-17 microRNAs (miRNAs) (Ibáñez *et al.*, 2018). Closely similar is the dsDNA genome of HSV-2 with a size of approximately 154,700 kb. At least 84 open reading frames (ORFs) are responsible for the protein-coding potential of the infectious particle (Minaya *et al.*, 2017). Characteristically, the numerous open reading frames (ORFs) of herpes simplex virus type 1 (HSV-1) overlap with the densely packed viral proteins (Chaitanya, 2019). According to the most recent ICTV database, members of the genus *Simplexvirus* are included in the group of viruses with class IV genome architecture.

The infectious genomes of human herpes simplex viruses (HSV-1 and HSV-2) consist of two covalently linked segments - a long (L) fragment occupying 82% of the genome, and a short (S) fragment with 18% of the genome (Mahiet *et al.*, 2012), which in turn arrange relative to each other by intramolecular recombination. The long (L) fragment consists of unique sequences (UL), and the short (S) fragment likewise consists of unique sequences (US) (Dolan *et al.*, 1998; Newman *et al.*, 2015). Each of the unique regions (UL and US) is flanked by a set of inverted repeats (TRL and IRL, TRS and IRS) (Strang and Stow, 2005). At the genomic termini, there are direct repeats of a sequence called the 'α' sequence, and, respectively, copies of inverted repeats designated the α' sequence located at the L-S junction fragment. The inverted copies of the α-sequences promote recombination between the termini and internal repeats, which in turn leads to subsequent inversion of the L and S components. This gene rearrangement leads to four viral genome isomers packaged into infectious particles (Newman *et al.*, 2015). The terminally truncated region of the HSV-1 genome ('α' sequence) contains all the *cis*-acting sequences required for DNA packaging. This region, which is most commonly 250 to 500 bp in length depending on the virus strain, is present as a single copy at the S-terminus, and as one or more tandem copies at the L-terminus. In addition, one or more copies are known to be present in an inverted orientation between the L and S segments at the L-S junction (Tong and Stow, 2010;

Hodge and Stow, 2001). During dsDNA replication, the inversion of L and S segments occurs at equal frequency in most wild-type HSV-1 populations (Chaitanya, 2019). Structurally, the 'α' sequence is flanked by direct repeats (DR1) of 19 to 20 bp, with a single copy of DR1 separating the 'α' sequence tandem. The genomic termini-generating event is characterized by cleavage toward one end of DR1, and circularization of infecting genomes restores a complete 'α' sequence. The central region of the 'α' sequence contains multiple repeats of one or two other short sequences (DR2 and sometimes DR4), while quasi-unique sequences lie between DR1 and the other direct repeats, DR2/DR4. These regions are known as Ub and Uc. Logistically, their location on the linear infectious genome is adjacent to the S and L termini (the Uc element is adjacent to the L terminus and the Ub is adjacent to the S terminus) (Hodge and Stow, 2001; Tong and Stow, 2010). The fragment (UC-DR1-Ub) contains 200 bp and spans the junction between the tandemly 'α' sequences generated by ligation of the genomic termini. This fragment contains all the essential *cis*-acting sequences necessary for viral DNA packaging. Within the Ub and Uc regions are two domains, *pac 1* and *pac 2*, respectively, which contain several sequence motifs specific for herpesviruses (Tong and Stow, 2010). The *pac 1* domain consists of a 3- to 7-bp adenine (A) or thymine (T)-rich region flanked by 5 to 7 nucleotides of cytosine (C). On the other hand, the *pac 2* motif consists of a 5- to 10-bp adenine (A)-rich region that is often associated with a nearby [CGCGCG] genomic sequence. Both *pac 1* and *pac 2* are located at opposite ends of dsDNA 30 to 35 bp from the genome termini (McVoy *et al.*, 1998).

Non-structural proteins (NSPs)

The members of the genus *Simplexvirus* encode numerous non-structural proteins (NSPs) responsible for the proper viral replication, establishment of latent infection, assembly of new infectious virions and their subsequent release from the infected host cell. Functionally, the non-structural proteins of both infectious agents of this taxon belong to 5 groups, each of which plays a key role in a different stage of the viral life cycle: viral replication complex, peripheral replication proteins, non-structural proteins (NSPs) involved in DNA-packaging, non-structural protein (NP) in the protein composition of the viral capsid, non-structural protein (NP) with control and modulating functions.

Gene	Gene Name	Function	References
Viral replication complex			
UL30	Catalytic subunit of DNA polymerase (Pol)	3'-5' exonuclease (Exo) activity and 5'-3' polymerase activity	Lawler and Coen, 2018; Terrell and Coen, 2012
UL42	Subunit for processivity of DNA polymerase	Stimulates viral genome synthesis	Jiang <i>et al.</i> , 2007
UL9	Ori-binding protein (OriBP)	Has two specific sites for binding to the double-stranded nucleic acid – OriS and OriL	Bazhulina <i>et al.</i> , 2013
UL5	5'-3' helicase subunit	DNA duplex unwinding for initiation of replication	Bermek and Williams, 2021
UL8	Primase subunit	Interacts with the other proteins in the complex (e.g. UL9, ICP8 and UL30) and modulates the enzymatic functions of the subcomplex UL5/UL52.	Chen <i>et al.</i> , 2007
UL52	DNA primase	Synthesizes short RNA primers to provide a substrate for DNA polymerase	Bermek and Williams, 2021; Bermek <i>et al.</i> , 2017
UL29	Single-stranded DNA-binding protein (SSB/ICP8)	Forms filaments during the formation of prereplicative sites and replication compartments; stimulates late gene transcription.	Darwish <i>et al.</i> , 2016; Bryant <i>et al.</i> , 2012
Peripheral replication proteins			
UL23	Thymidine kinase (TK)	Involved in the nucleoside salvage pathway; catalyzes the transfer of the γ -phosphate of ATP to dT, forming dTMP.	Xie <i>et al.</i> , 2019; Kokoris and Black, 2002
UL39	The large subunit of ribonucleotide reductase (ICP6)	Inhibits apoptosis and activates or inhibits necroapoptosis; Required for replication in neurons and establishment of latent infection; Together with UL40 mediates the conversion of NTPs to dNTPs.	Mostafa <i>et al.</i> , 2018; Torrents, 2014
UL40	The small subunit of ribonucleotide reductase	Together with UL39 mediates the conversion of NTPs to dNTPs.	Mostafa <i>et al.</i> , 2018; Torrents, 2014
UL50	Deoxyuridine 5'-triphosphate nucleotide hydrolase (dUTPase)	Maintains a low level of dUTP by converting dUTP to dUMP.	Sire <i>et al.</i> , 2008
UL2	Uracil DNA glycosylase (UDG)	Removes uracil (U) from DNA molecule, reducing the possibility of point mutations; Reactivates HSV-1 infections in neurons	Cai <i>et al.</i> , 2020; Sire <i>et al.</i> , 2008
Non-structural proteins (NSPs) participating in DNA packaging			
UL12	5'-3' exonuclease / type I alkaline nuclease (Exo)	5'-to-3' exonuclease activity; Participates in the production of mature packaged unit-length linear progeny viral DNA molecules; Promotes efficient SSA.	Grady <i>et al.</i> , 2017; Corcoran <i>et al.</i> , 2009; Schumacher <i>et al.</i> , 2012
UL15	Terminase large subunit	Responsible for viral DNA cleavage and packaging during the final stage of the viral life cycle; They provide the energy for DNA translocation.	Yang <i>et al.</i> , 2011; Heming <i>et al.</i> , 2014
UL28	Terminase small subunit	Binds nucleotide sequences responsible for viral DNA packaging.	Yang <i>et al.</i> , 2011

Gene	Gene Name	Function	References
UL6	Portal protein	Mediates DNA entry into the capsid through a unique portal vertex composed of 12 copies of the UL6 protein.	Yang <i>et al.</i> , 2011
UL25	Capsid protein	Located on the capsid surface in a proposed heterodimer with capsid vertex protein component 1 (UL17/CVC 1).	Cockrell <i>et al.</i> , 2009
UL 32	Packaging protein	Essential for the cleavage of concatemeric viral DNA into monomeric genomes and their packaging into preformed capsids.	Palmer, 2010
UL33	DNA packaging protein	Forms stable association with UL28.	Yang <i>et al.</i> , 2011
UL17	Capsid vertex protein component 1 (CVC1)	Reinforce capsid stability.	Wang <i>et al.</i> , 2018
Non-structural protein (NP) in the protein composition of the viral capsid			
UL26	Protease	Also catalyses scaffold protein binding to capsid.	Sheaffer <i>et al.</i> , 2000
UL26.5	Capsid protein	Effectuates the connection between the formed scaffold and the capsid.	Sheaffer <i>et al.</i> , 2000
Non-structural protein (NP) with control and modulating functions			
UL13	Serine/threonine protein kinase (Us3)	Involved in viral gene expression in infected cells; Functions as a positive regulator of viral replication; Involved in nucleocytoplasmic transport of nucleocapsids across nuclear membranes, and increases gene expression by blocking histone deacetylation.	Kato <i>et al.</i> , 2006; Shindo <i>et al.</i> , 2016
UL54	Major mRNA export factor	Regulates viral gene expression through several different mechanisms, including splicing, mRNA 3' processing, and mRNA export; Disrupts transcription termination in host genes.	Wang <i>et al.</i> , 2019; Wang <i>et al.</i> , 2020

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