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HERPES SIMPLEX VIRUS

Travis J Taylor, Mark A. Brockman, Elizabeth E. McNamee, and David M. Knipe

Harvard Medical School, Department of Microbiology and Molecular Genetics, 200 Longwood Avenue, Boston, MA 02115

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1. ABSTRACT

Herpes simplex virus (HSV) commonly causes human infections in the orofacial region (HSV-1) and in the genital region (HSV-2). Productive viral infection in mucosal epithelial cells may result in clinical symptoms and is followed by a latent infection within sensory neurons. During productive infection a large number of viral gene products are expressed while during latent infection few or no viral proteins are expressed. Reactivation from latency results in recurrent infections and disease at or near the primary site of infection. Understanding the details of the two stages of the HSV life cycle is a particular focus of current research on HSV. The virus interacts with and modifies numerous host cell functions in both epithelial and neuronal cells, and studies of HSV have enhanced our knowledge of many fundamental processes in eukaryotic cells. Ongoing research continues to uncover novel effects of HSV on cells, and a complete understanding of HSV infection during both productive and latent infection should allow the design of new antiviral agents and vaccines and increased knowledge of basic cell and molecular biology. This review article is designed to provide an introduction to HSV biology and key aspects of the infection cycle.

2. INTRODUCTION

Herpes simplex virus (HSV) is a common human pathogen, causing infections of orofacial mucosal surfaces (HSV-1) and genital mucosal surfaces (HSV-2). Productive infection results in the formation of vesicular lesions in the mucosal epithelia, followed by spread of the virus to sensory neurons and establishment of a latent infection that may remain for the life of the host. Reactivation of dormant virus results in recurrent disease at or adjacent to the site of primary infection. The common cold sores caused by HSV-1 and the genital herpes lesions caused by HSV-2 are not life-threatening conditions, but serious pathology can result from infections of the cornea (keratitis) or central nervous system (encephalitis), and infection of newborns or immunocompromised individuals can result in severe disseminated disease (1).

During productive infection, HSV takes over the host cell and directs a new transcriptional program of viral gene expression (2). Extensive investigation of HSV infection has provided several paradigms of eukaryotic molecular biology. One of the first functional eukaryotic promoters was defined through studies of the HSV thymidine kinase (TK) gene (3), and the HSV virion protein VP16 is one of the best characterized and most widely used transcriptional transactivators containing an acidic activation domain (4, 5). Because of its large genome size, HSV encodes a large number of proteins with discrete modulatory functions. These viral factors regulate the infectious process at multiple stages, including gene expression, DNA synthesis, and virion assembly. Numerous genes have been identified to be essential for viral replication based on *in vitro* assays, although a great number of HSV's predicted 84 proteins may be required in the context of a particular *in vivo* niche. This review will present the following key elements of HSV biology: 1) virion structure; 2) genome organization; 3) the stages of productive virus infection, including entry, gene

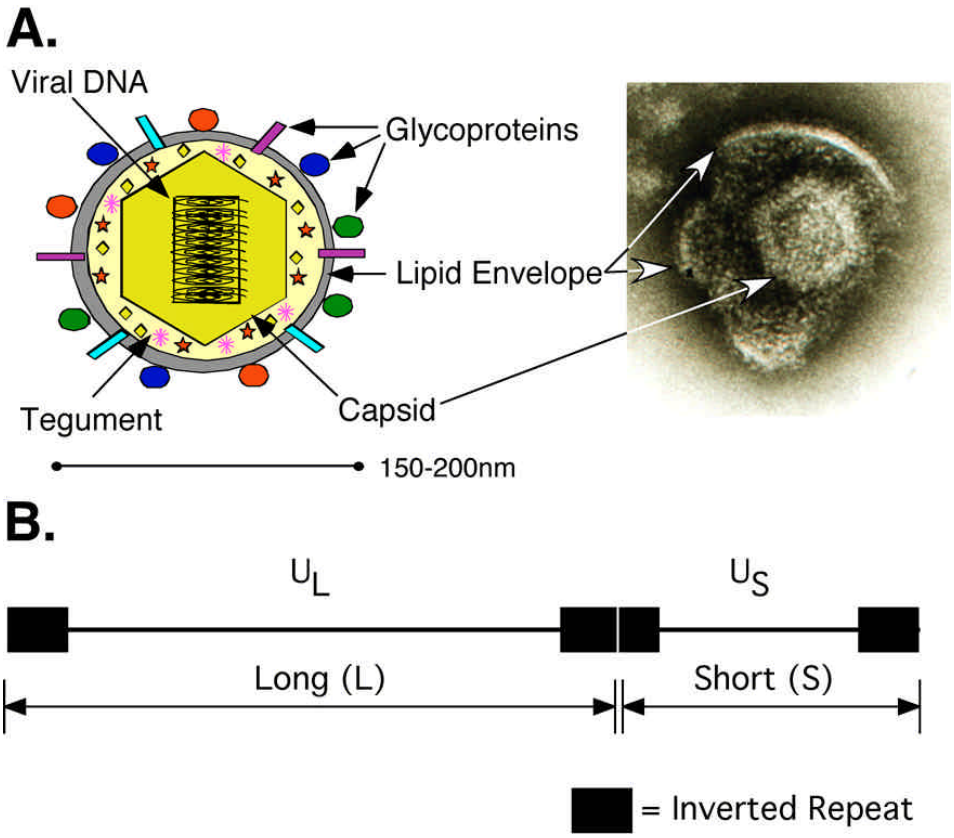

Figure 1. HSV virion structure and genome organization. The HSV virion (A) is comprised of four major features: (1) the viral double-stranded DNA, packaged as a tightly wrapped spool; (2) an icosahedral capsid shell; (3) a tegument layer containing numerous viral proteins; and (4) a lipid membrane envelope studded with viral glycoproteins. The major structural components of the virion can be seen in an EM photograph (right) where the viral envelope is ruptured and folded back. The HSV genome (B) is organized into two components, long (L) and short (S). Each component contains a unique region (U) flanked by inverted repeat regions and are linked to generate a total genome size of approximately 150,000 base pairs.

expression, DNA synthesis, and virion assembly and egress; and 4) latent infection.

3. VIRION STRUCTURE

HSV-1 and HSV-2 are the common or vernacular names for the species formally named human herpesvirus 1 and human herpesvirus 2, respectively. They are classified in the genus *Simplexvirus* in the subfamily *Alphaherpesvirinae* in the family *Herpesviridae* by the International Committee on the Taxonomy of Viruses (ICTV). HSV is a large (150-200nm diameter) enveloped virus (2) with a distinct virion structure characteristic of the herpesviruses **(**Figure 1A). A mature HSV particle contains four structural features (6): 1) an electron-opaque core containing viral DNA, 2) an icosahedral capsid, 3) an intermediate phase or 'tegument' that contains additional viral proteins, and 4) an outer membrane envelope studded with viral glycoprotein spikes. A linear, dsDNA viral genome (7, 8, 9) is packaged within the viral capsid shell as a tightly wrapped toroid structure (10). Additional viral proteins are associated with the capsid and a lipid membrane envelope surrounds this complex (11).

4. GENOME ORGANIZATION

The HSV genome (GenBank Accession # X14112 for the HSV-1 sequence and #Z86099 for the HSV-2 sequence) consists of approximately 150,000 base pairs (8). This large genome allows the virus to encode at least 80 gene products. The genome is composed of two covalently linked segments, called Long (L) or Short (S), based upon their relative length (Figure 1B). These segments each contain unique sequence regions (U_L or U_S) flanked by inverted repeat sequence (12). The genes found in the unique regions are present in the genome as a single copy, but genes that are encoded in the repeat regions, such as ICP0 or ICP4, are present in the genome in two copies. Viral genes (and their protein products) are generally named by their relative position from left to right in the U_L or U_S region (for example: U_L1, U_L2 or U_S1, U_S2). However, many viral proteins are also described by alternate names based upon their functions, Infected Cell Protein (ICP) number, Virion Protein (VP) number, or molecular weight. This can be confusing because many viral proteins are identified by multiple names in the literature. To assist the reader, alternate names of many gene products have been designated in this chapter where applicable.

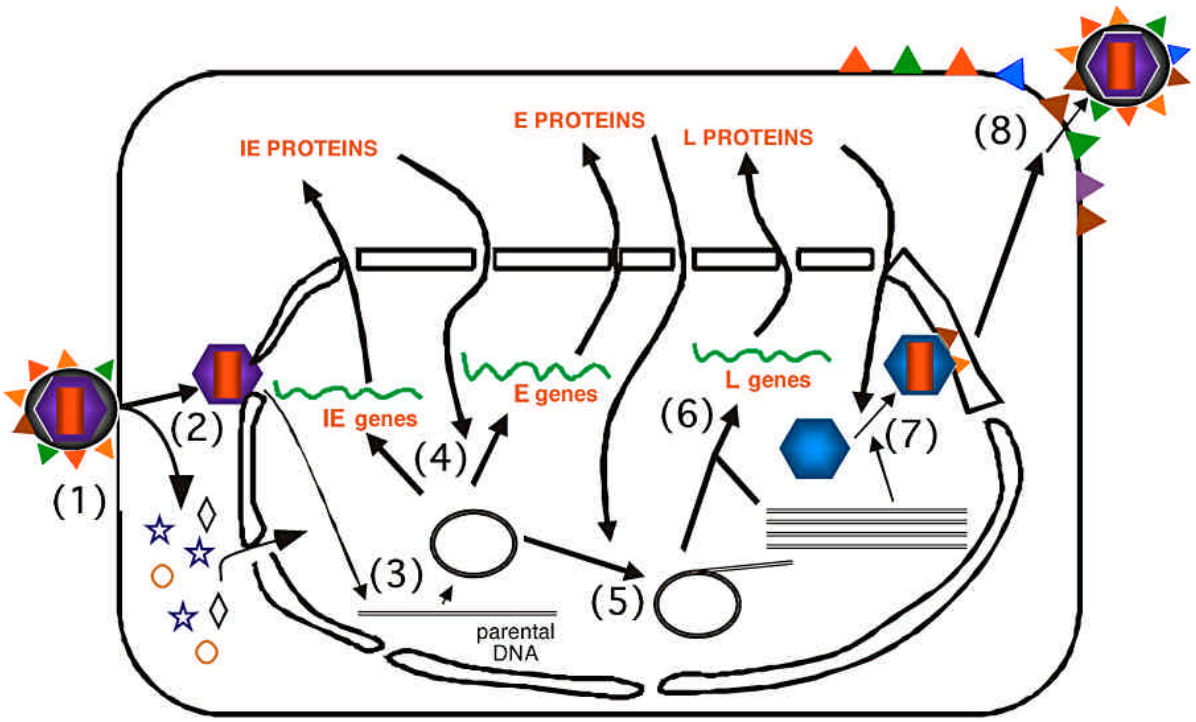

Figure 2. The cycle of productive HSV replication in a cell. The stages of HSV infection are: (1) Receptor binding and membrane fusion; (2) Release of the viral nucleocapsid and tegument into the cell cytoplasm and transport of the nucleocapsid to the nuclear pore; (3) Release of viral DNA into the nucleus; (4) Transcription and translation of the viral immediate early (IE) and early (E) genes; (5) viral DNA synthesis; (6) Transcription and translation of the viral late (L) genes; (7) capsid assembly and DNA packaging; and (8) egress of progeny virions.

HSV contains three origins of replication within the genome that are named depending upon their location in either the Long (*ori*L) or Short (*ori*S) region of the genome. *Ori*L is found as a single copy in the U_L segment, but *ori*S is located in the repeat region of the Short segment; thus, it is present in the genome in two copies. Both *ori*L (13, 14) and *ori*S (15, 16) are palindromic sequences consisting of an AT-rich center region flanked by inverted repeats that contain multiple binding sites of varying affinity for the viral origin binding protein (U_L9). Either *ori*L or one of the *ori*S sequences is sufficient for viral replication (17, 18).

The viral genome also contains signals that orchestrate proper processing of the newly synthesized genomes for packaging into pre-formed capsids. Progeny genomes are generated in long concatemers that require cleavage into unit-length monomers. For this purpose, the viral genome contains two DNA sequence elements, *pac1* and *pac2*, that ensure proper cleavage and packaging of unit-length progeny genomes. These elements are located within the direct repeats (DR) found within the inverted repeat regions at the ends of the viral genome.

5. STAGES OF PRODUCTIVE VIRUS INFECTION

Productive infection of a cell by HSV involves several key steps (shown in Figure 2), including entry, viral gene expression, viral DNA synthesis, and assembly/egress of progeny virions (2).

6. VIRUS ENTRY

Entry of HSV into a cell proceeds in a step-wise fashion and involves the function of a number of viral envelope proteins, including at least glycoprotein B (gB), gC, gD, and the gH/gL heterodimer. First, virus attachment is initiated by interactions between virion gC and heparan sulfate moieties on the host cell (19). While this initial step enhances infection, it is not an absolute requirement because gC is dispensable for viral growth and replication *in vitro* (19) and cells that do not express heparan sulfate remain permissive at low levels (20, 21). Second, virion attachment is stabilized by interactions between the gD protein and one of a number of recently identified cellular receptors, collectively referred to as Herpes virus entry (Hve) proteins (22). These cellular receptors are members of two larger protein families: 1) the tumor necrosis factor (TNF) family, including HveA (23, 24); and 2) the immunoglobulin superfamily, including HveB (25), HveC (also known as nectin-1α) (26), and HIgR (also known as nectin-2α) (27). Finally, the viral envelope fuses with the cellular membrane by an undetermined mechanism that appears to require gD (28), gB (29), and the gH/gL heterodimer (30). Following fusion, the viral nucleocapsid and tegument proteins are released into the cytoplasm of the host cell. The nucleocapsid and some tegument proteins (VP16, VP1-2) are transported to the nucleus via microtubules (31) while other tegument proteins remain in the cytoplasm (vhs,U_S11). Empty viral capsids have been observed accumulating at the nuclear envelope following infection and associate with nuclear pore structures (32).

Table 1. Immediate early gene products involved in regulation of gene expression

Protein name	Essential	Functions
ICP0	NO	• Promiscuous transactivator of all classes of HSV genes (44, 45, 46, 47) • Essential for infection at low MOI (48) • Binds to cellular proteins involved in protein degradation • Has E3 ubiquitin ligase activity (49) • Disrupts cellular ND10 sites (50, 51)
ICP4	YES	• Essential for the transcription of all early and late genes (52, 53) • Negatively regulates its own expression by binding to consensus sites within the ICP4 promoter (54, 55)
ICP27	YES	• Essential for late gene transcription (56) • Essential for high level expression of some early genes (57) • Redistributes snRNPs and inhibits splicing (58, 59, 60) • Shuttles between nucleus and cytoplasm (61, 62)
ICP22	NO	Required for: • Modification of the host RNA polymerase II (63) • Optimal expression of ICP0 (64) • High expression of some late genes (65)

7. VIRAL GENE EXPRESSION

DNA viruses utilize the host cell in a variety of ways to express the viral proteins necessary for viral replication and spread. Generally, the virus must express two types of proteins, non-structural proteins necessary for viral DNA replication and structural proteins that make up the virion and envelope for packaging the viral DNA. All herpes viruses share the characteristic of a temporally regulated cascade of gene expression (33). Herpes simplex virus genes are expressed in 3 temporal classes: immediate early (α), early (ß) and late (γ) genes. While these classes are convenient for use in nomenclature, it should be noted that the progression of viral gene expression is likely to take place gradually, rather than in distinct stages. Immediate early genes are transcribed first after infection and activate transcription of the early genes, whose gene products replicate the viral DNA. Viral DNA replication stimulates the expression of the late genes, encoding the structural proteins.

After viral DNA enters the nucleus, transcription of the viral genes begins. Host RNA polymerase II is responsible for synthesis of all viral mRNAs (34, 35). While cellular proteins are sufficient for the synthesis of viral transcripts, viral proteins are necessary for the initiation and enhancement of transcription of certain genes. There are a number of essential viral proteins involved at each of these steps. These proteins act in concert with an abundance of cellular proteins to produce the full range of viral gene products needed for productive viral infection.

The first genes transcribed during viral infection are the immediate early (IE) or α genes. Initiation of transcription of these genes proceeds by recruitment of cellular transcriptional machinery to IE gene promoters that contain numerous host regulatory sequences. IE gene expression does not require prior HSV protein synthesis. The virion protein VP16, however, plays an important role in enhancing the expression of the IE proteins (36). VP16 is known to interact with a number of cellular proteins, including HCF, Oct-1, TFII-B and TFII-D (37, 38, 39, 40, 41, 42). It is thought that the interaction between VP16 and HCF in the cytoplasm causes the translocation of VP16 into the nucleus (43), where it binds to the Oct-1 already bound to viral DNA via consensus TAATGArATT sites upstream of the RNA cap site. This enhances the activity of the cellular transcriptional machinery already assembled on the IE gene promoters. IE proteins include several multifunctional proteins that play essential roles in the regulation of later viral gene expression as well as in control of the host cell (Table 1).

Immediate early proteins ICP4, ICP27, and ICP0 promote the transcription of the early (E) genes. Early gene promoters contain binding sites for a variety of cellular transcription factors, and it is thought that ICP4 activates E gene expression by interacting with some of these transcription factors (66, 67, 68). ICP27 also plays a role in the synthesis of some E proteins, but it is not known whether ICP27 acts at the transcriptional or posttranscriptional level to exert this function (57, 69, 70). ICP0 acts in concert with cellular proteins to both activate transcription and to alter the host cell to create an environment conducive to viral protein synthesis and DNA replication. The E proteins are generally involved in viral DNA replication and are described in detail below. One early protein with a role in transcriptional regulation is the viral ssDNA binding protein, ICP8. ICP8 is necessary for transcription of late genes beyond its role in DNA replication (71, 72). This may involve rearrangement of the viral genome to allow late-specific transcription factors access to the promoters or initiation of a switch to transcription from progeny genomes (73).

Prior to DNA replication, IE proteins initiate the transcription of not only the E genes, but also a subset of the late genes, called early/late, leaky late, or γ_1 genes. The synthesis of these proteins, while later in the infection cycle, is not strictly dependent on viral DNA replication. However, their levels are significantly enhanced upon initiation of DNA replication. A second subset of late genes, the true late or γ_2 genes, is transcribed only after the initiation of viral DNA replication. The promoters of these

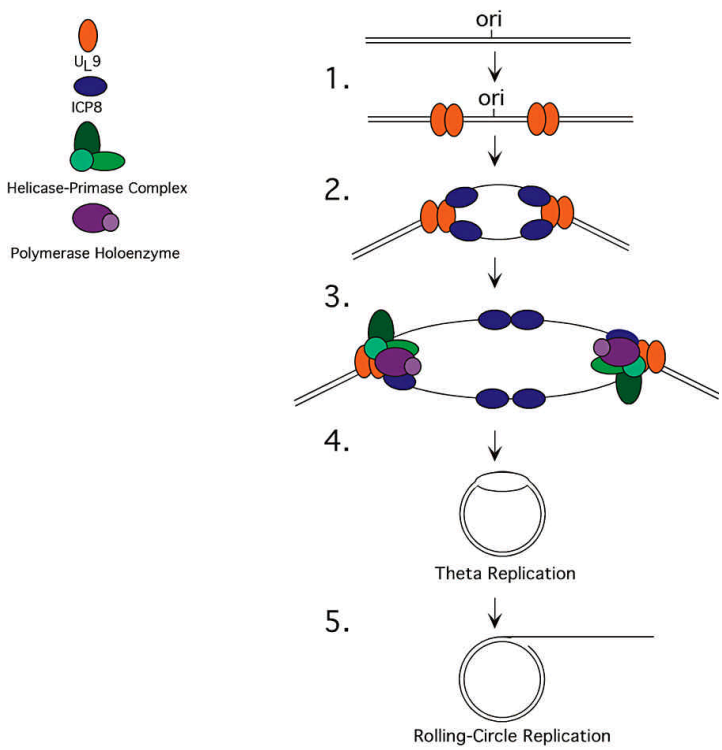

Figure 3. Diagram of HSV DNA Replication. 1) The origin binding protein, UL9, binds to specific sites at an origin (either *ori*L or *ori*S) and starts to unwind the DNA. 2) The single-stranded DNA binding protein, ICP8, is recruited to the unwound DNA. 3) UL9 and ICP8 recruit the five remaining replication proteins to the replication forks. 4) DNA synthesis initially proceeds via a theta replication mechanism, but then switches to a rolling-circle replication mechanism (5).

genes do not provide much evidence about the control of their transcription, as there are no specific sequences recognizable by viral transcription factors.

One of the important regulatory events in the cascade of gene expression is the shutdown of immediate early and early gene expression. There are two defined examples of this. ICP4 represses itself by binding to repressor elements in its own promoter (54, 55, 74, 75). The decrease in ICP4 levels leads to a reduction of early and late gene expression, as ICP4 is essential for those functions. ICP8 plays a role in reducing transcription from the parental genome following viral DNA replication (76, 77, 78). It is thought that one of the major triggers between early and late gene transcription is a movement of the transcription machinery from the parental genome to the progeny genomes. Therefore, if transcription from the parental genome is limited, immediate early and early gene expression is reduced.

In addition to stimulating the expression of its own proteins, HSV shuts down host cell RNA, DNA and protein synthesis (79). Herpes simplex virus uses numerous cellular proteins during its gene expression. Presumably, to gain access to these proteins, the virus disrupts host transcription, replication, and translation. It annexes these cellular components for use in the synthesis of its own proteins.

HSV disrupts the cell in at least three ways. First, the virion vhs protein causes the degradation of existing mRNAs early in infection (80, 81, 82, 83, 84). Next, multiple viral genes are involved to impede cellular transcription and translation (85, 86, 87). In one example, ICP27 inhibits RNA maturation by redistributing splicing factors (59, 60, 89). Because very few viral transcripts are spliced, this gives the viral mRNAs a competitive advantage for translational machinery. Another protein, ICP22, has been shown to be required for modification of host RNA polymerase II following infection (88), perhaps altering the ability of this complex to transcribe from the viral genome. Finally, the virus selectively destabilizes a variety of cellular proteins, especially those involved in regulation of the host cell cycle (90, 91, 92).

8. VIRAL DNA REPLICATION

HSV DNA replication occurs in specialized structures formed in the nucleus of infected cells that have been called replication compartments (93). Viral DNA (94) and viral DNA replication proteins (57) are localized adjacent to cellular structures called ND10 sites, and replication compartments form at these sites as viral DNA synthesis proceeds. The linear input viral DNA circularizes upon entry into the nucleus (57). Viral DNA replication initiates at one of the three origins of replication within the HSV genome and is believed to proceed initially via a theta replication mechanism (Figure 3). Once DNA synthesis begins, it is likely that a rolling-circle replication mechanism takes over to produce the majority of the progeny genomes in an infected cell (95). Seven herpes

Table 2. Essential HSV DNA replication proteins

Protein Name	Gene	Activity
Origin-binding protein	U_L9	• Binds HSV origins (*ori*L and *ori*S) • DNA helicase activity
Single-stranded DNA-binding protein (ICP8)	U_L29	• Binds ssDNA • Stimulates DNA polymerase and helicase-primase activities
Helicase-primase complex	U_L5/U_L52	• DNA helicase activity • DNA primase activity
	U_L8	• Stimulates activities of U_L5/U_L52 complex
DNA polymerase holoenzyme	U_L30	• DNA polymerase activity • Exonuclease activity
	UL42	• DNA polymerase accessory subunit • Promotes processive DNA synthesis

genes (Table 2) were identified by genetic and biochemical methods as essential for viral DNA replication (96). These seven genes encode protein products that function as an origin binding protein (U_L9) (97), a single-stranded DNA binding protein ICP8 (U_L29) (98), a helicase-primase complex ($U_L5/U_L8/U_L52$), and a DNA polymerase (U_L30/U_L42) (99). While the virus encodes many protein products required for viral DNA synthesis, other cellular factors, such as DNA ligases and topoisomerases, may also function in the viral DNA replication process.

Origin Binding Protein (U_L9, OBP). The origin binding protein is an 851 amino acid residue protein that contains ATP-binding and DNA helicase motifs, both of which are required for viral DNA replication. U_L9 binds specifically as a homodimer to sites in the origin of replication containing the DNA sequence CGTTCGCACTT (100, 101). The binding of U_L9 to the origin is believed to cause a bend in the DNA allowing for the distortion of the DNA structure to form a single-stranded stem loop structure. The ssDNA and U_L9 may recruit the single-stranded DNA binding protein, ICP8, which in turn stimulates the helicase activity of U_L9.

Single-Stranded DNA Binding Protein (U_L29, ICP8). The major HSV DNA binding protein is a large, multi-functional protein that consists of 1196 amino acid residues. ICP8 binds preferentially to single-stranded DNA in a cooperative manner (102, 103). ICP8 is required for viral DNA synthesis and the efficient expression of late genes. In addition to its cooperative DNA binding activity, ICP8 has a DNA helix-destabilizing activity (104) and can also promote renaturation of complementary single DNA strands. ICP8 has been shown to interact with and stimulate the helicase activity of U_L9 (105), promote the helicase-primase activities of the helicase-primase core subunit U_L5/U_L52 in the presence of U_L8 (106, 107), and modulate the polymerase activity of U_L30 (108). The ability of ICP8 to interact either physically or functionally with several other viral replication proteins, and possibly cellular proteins, supports the notion of ICP8 acting as a "scaffolding protein" for viral DNA replication which acts to recruit replication proteins to the correct site in the nucleus. This is also supported by the fact that ICP8 is required for the formation of viral replication compartments in the nucleus of infected cells (109, 110).

Helicase-Primase Complex, ($U_L5/U_L8/U_L52$). The helicase-primase complex is a heterotrimer composed of the U_L5, U_L8, and U_L52 gene products (111). The 882 amino acid residue U_L5 protein contains conserved ATP-binding and helicase motifs. The 1058 amino acid residue U_L52 protein has a divalent metal binding motif that is found in other DNA primases. The core helicase, primase, and ATP-ase enzymatic activities are contained in a sub-complex of U_L5 and U_L52. The presence of the 750 amino acid residue U_L8, along with ICP8, stimulates the core enzymatic activities. The helicase activity has a 5'-3' activity.

DNA Polymerase Holoenzyme (pol, U_L30/U_L42). The DNA polymerase holoenzyme is a heterodimer consisting of the 1235 amino acid residue catalytic subunit (U_L30) and its 488 amino acid residue accessory factor (U_L42) (112). U_L30 serves as the polymerase as shown by its homology with other known DNA polymerases (113). U_L30 also has an intrinsic 3'-5'-exonuclease activity, which allows for proof-reading while the polymerase is copying the DNA. The presence of U_L42 increases the processivity of U_L30. The interaction between U_L30 and U_L42 is required for viral DNA replication.

The DNA polymerase holoenzyme has served as a target for the development of anti-viral compounds. One reason for this is the broad substrate specificity exhibited by the catalytic U_L30 subunit compared to other cellular polymerases. Another target is the specific, essential interaction between U_L30 and U_L42.

In addition to the seven essential viral replication proteins, HSV expresses several other early viral gene products, such as thymidine kinase (U_L23) (114), ribonucleotide reductase (U_L39/U_L40) (115), and uracil N-glycosylase (U_L2) (116). These proteins are considered "nonessential" for viral DNA replication. This term, however, may be somewhat misleading. While these gene products are dispensable for replication in some cell culture models, they are required for growth in certain tissues *in vivo* or for productive infection of non-dividing cells. Many of the "nonessential" genes found in HSV encode protein products that function in nucleotide metabolism. These activities may be critical *in vivo* because nucleotide pools, which are present in abundance in actively dividing

cells, are limiting in resting cells, such as neurons. As such, these proteins may give the virus a growth advantage either during productive infection in certain tissues or during reactivation from latency in neurons. The thymidine kinase protein is a popular target for the development of anti-viral therapies. In addition to its normal role of phosphorylating nucleosides for use in viral DNA synthesis, thymidine kinase will phosphorylate, and thus activate, anti-viral nucleoside analog drugs, such as acyclovir (117). These analogs are then incorporated into viral DNA genomes.

9. VIRION ASSEMBLY, DNA ENCAPSIDATION, AND EGRESS

Assembly of the viral capsid requires synthesis of numerous late proteins and occurs within the nucleus. Because some capsid proteins lack nuclear localization sequences (NLS) (i.e. VP5, VP26, VP23), these proteins must form complexes with NLS-containing proteins (i.e. VP19C or pre-VP22a) in the cytoplasm for transport into the nucleus. A mature HSV capsid is comprised of an outer shell containing penton-shaped subunits of the major capsid protein, VP5, and hexons of VP5 and VP23 (118, 119). These subunits are connected by triplex structures formed by the two minor capsid proteins, VP19C (U_L38) in one copy and VP23 (U_L18) in two copies. Two non-structural genes encoding multiple proteins, U_L26 (VP21 and VP24) and $U_L26.5$ (pre-VP22a, VP22a), are also necessary for efficient capsid formation. VP21, pre-VP22a, and VP22a are scaffolding proteins. VP24 is a serine protease required for capsid maturation. Capsid assembly does not require any cellular factors and can be completed *in vitro* using purified viral protein components (120).

Empty capsid shells are loaded with viral DNA by a process that simultaneously resolves concatemers and packages genome-length monomers within the capsid (121, 122) to form a nucleocapsid. The mechanism of DNA cleavage and packaging is not well understood; however, it is known to require site-specific breaks to the concatemers at specific distances from the *pac1* and *pac2* sites (123, 124). This process also requires numerous viral gene products, including U_L6, U_L15, U_L25, U_L28 (ICP18.5), U_L32, U_L33, U_L36 (ICP1-2), and U_L37.

The route of virion egress from an infected cell remains somewhat controversial. It has been observed that the nucleocapsid can bud through the inner nuclear membrane (125), acquiring some tegument proteins and a glycoprotein studded envelope in the process, but two pathways have been proposed from this point. A re-envelopment model (126, 127) suggests that enveloped virions fuse with the outer nuclear membrane, thereby releasing free nucleocapsids into the cytoplasm that re-envelope by budding into the Golgi compartment. These re-enveloped particles are secreted from the cell by a vesicular route. A lumenal pathway model (128) proposes that enveloped virions traffic from the inner nuclear space to the Golgi in vesicles or within the lumen of the endoplasmic reticulum (ER), and are released from the cell by a normal secretory route. Recent evidence using electron microscopy and ER-retrieved glycoproteins lends considerable support to the re-envelopment model as the major route of virus egress (129, 130).

10. LATENT INFECTION

One of the hallmarks of all herpesvirus infections is the ability of the virus to establish a latent infection that can last the lifetime of the host. Unlike a persistent infection, in which the virus is constantly replicating at some level, during HSV latency, no viral progeny are produced and only very limited gene transcription is detected. The major site of HSV latent infection is sensory neurons in ganglion tissue, either trigeminal ganglia for HSV-1 or sacral ganglia for HSV-2. After the initial primary infection, generally at an oral or genital mucosal surface, the virus travels along the innervating neuronal axon to the neuronal cell body (Figure 4A). Once within the neuron, the virus enters a quiescent state in which the lytic gene products are not produced. During latency, the HSV genome remains in the nucleus of the neuron as circular, extra-chromosomal DNA (131, 132). In neurons latently infected with HSV, the only abundant viral RNA detected is a family of transcripts referred to as the latency-associated transcripts or LATs, which will be discussed in greater detail below. This transcriptional silence may allow the virus to remain hidden in the cell by avoiding immune surveillance. The virus remains in this state for the lifetime of the host, or until the proper signals reactivate the virus and new progeny are generated. Progeny virus then travel through the neuron axis to the site of the primary infection to re-initiate a lytic replication cycle (133) (Figure 4B). The signals and mechanisms involved in this process are poorly understood, but it appears that certain physical stresses, such as illness or exposure to ultraviolet light, increase the chance of reactivation.

Latency Associated Transcripts (LATs). The LATs are the only major viral transcripts detected in latently infected neurons. The full-length 8.3-kilobase (kb) primary LAT transcript accumulates to low levels in latently infected neurons. The 2.0-and 1.5-kb introns derived from the full length LAT, however, are abundant and very stable (134, 135, 136). The role of LATs in latency is not well understood, partly because the LATs are not absolutely required for the establishment and maintenance of latent infection or reactivation in most animal model systems. While no LAT-encoded protein has been conclusively demonstrated to date, the LAT transcripts may have other functions or activities. It has been shown that LAT negative viruses have increased immediate early gene expression in neurons, suggesting that LATs may limit viral gene expression (137) and promote a latent state. It has been hypothesized that LATs act by an antisense mechanism because a portion of the 8.3-kb LAT partially overlaps with the ICP0 and ICP4 mRNAs (138). An alternate view is that LATs may protect neurons from apoptosis, thus allowing for neuronal survival during latent infection (139). Several views on the function of the LATs exist; thus, the fundamental mechanisms by which viral gene expression is restricted in neurons remain to be elucidated.

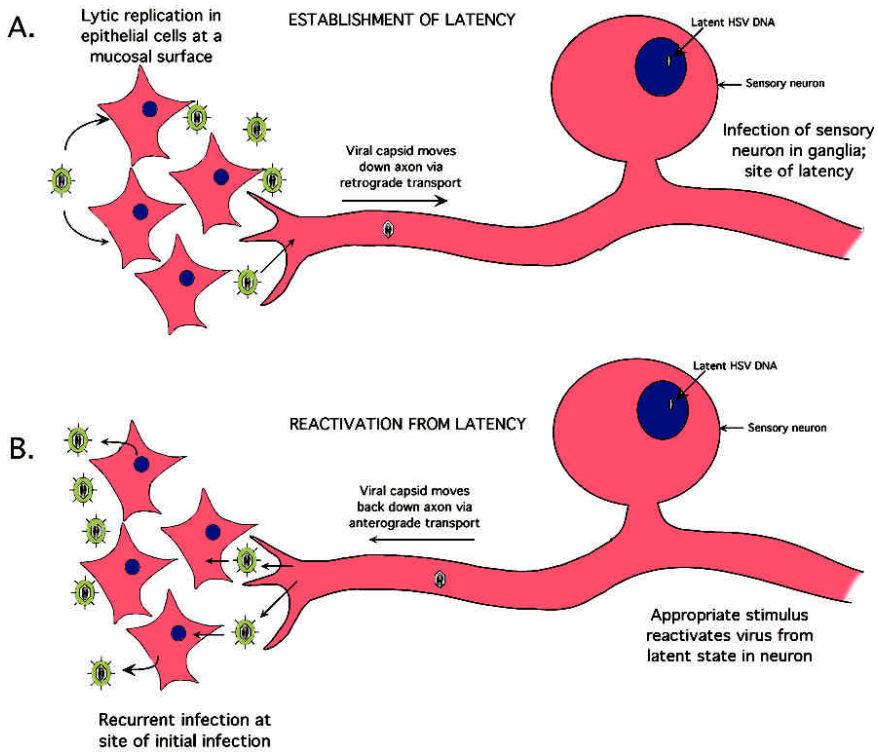

Figure 4. Stages of HSV infection. (A) HSV replicates initially in epithelial cells at the genital or oral mucosal surface. The virus enters innervating sensory neurons where it travels up the axon to the neuronal cell body. Once in the neuron, the virus establishes a latent infection that may last the lifetime of the host. (B) Upon the proper stimulus, the virus can reactivate, travel back down the axon, and establish another round of lytic replication.

11. SUMMARY AND PERSPECTIVE

The rudimentary outlines of the mechanisms of productive infection of epithelial cells and latent infection of neurons by HSV have been defined. Clearly, much more knowledge must be gained before we understand how this virus behaves so differently in these two cell types. Nevertheless, this challenge is of great significance because new information may lead to better antiviral drugs, more productive vaccines, more effective viral vaccine and gene therapy vectors, and increased knowledge of gene regulatory mechanisms in differentiated cells.

12. REFERENCES

1. R. J. Whitley, B. Roizman: Herpes simplex virus infections. *Lancet* 357, 1513-8 (2001)
2. B. Roizman, D. M. Knipe: Herpes simplex viruses and their replication. In: Fields Virology. Eds: D. M. Knipe, P. M. Howley, 4th ed, vol. 2. Lippincott, Williams & Wilkins, Philadelphia, PA 2399-2460 (2001)
3. S. L. McKnight, E. R. Gavis, R. Kingsbury, R. Axel: Analysis of transcriptional regulatory signals of the HSV thymidine kinase gene: identification of an upstream control region. *Cell* 25, 385-398 (1981)
4. S. J. Triezenberg, R. C. Kingsbury, S. L. McKnight: Functional dissection of VP16, the trans-activator of herpes simplex virus immediate early gene expression. *Genes & Development* 2, 718-729 (1988)
5. I. Sadowski, J. Ma, S. Triezenberg, M. Ptashne: GAL4-VP16 is an unusually potent transcriptional activator. *Nature* 335, 563-564 (1988)
6. P. Wildy, W. C. Russell, R. W. Horne: The morphology of herpes virus. *Virology* 12, 204-222 (1960)
7. Y. Becker, H. Dym, I. Sarov: Herpes simplex virus DNA. *Virology* 36, 184-192 (1968)
8. E. D. Kieff, S. L. Bachenheimer, B. Roizman: Size, composition, and structure of the deoxyribonucleic acid of herpes simplex virus subtypes 1 and 2. *Journal of Virology* 8, 125-132 (1971)
9. G. Plummer, C. R. Goodheart, D. Henson, C. P. Bowling: A comparative study of the DNA density and behavior in tissue cultures of fourteen different herpesviruses. *Virology* 39, 134-137 (1969)
10. D. Furlong, H. Swift, B. Roizman: Arrangement of herpesvirus deoxyribonucleic acid in the core. *Journal of Virology* 10, 1071-1074 (1972)
11. Z. H. Zhou, D. H. Chen, J. Jakana, F. J. Rixon, W. Chiu: Visualization of tegument-capsid interactions and DNA in intact herpes simplex virus type 1 virions. *Journal of Virology* 73, 3210-3218 (1999)
12. S. Wadsworth, R. J. Jacob, B. Roizman: Anatomy of herpes simplex virus DNA. II. Size, composition, and arrangement of inverted terminal repetitions. *Journal of Virology* 15, 1487-1497 (1975)
13. D. Lockshon, D. A. Galloway: Cloning and characterization of oriL2, a large palindromic DNA replication origin of herpes simplex virus type 2. *Journal of Virology* 58, 513-521 (1986)
14. S. K. Weller, *et al.*: Cloning, sequencing, and

functional analysis of oriL, a herpes simplex virus type 1 origin of DNA synthesis. *Molecular & Cellular Biology* 5, 930-942 (1985)
15. N. D. Stow: Localization of an origin of DNA replication within the TRS/IRS repeated region of the herpes simplex virus type 1 genome. *EMBO Journal* 1, 863-867 (1982)
16. N. D. Stow, E. C. McMonagle: Characterization of the TRS/IRS origin of DNA replication of herpes simplex virus type 1. *Virology* 130, 427-438 (1983)
17. M. Polvino-Bodnar, P. K. Orberg, P. A. Schaffer: Herpes simplex virus type 1 oriL is not required for virus replication or for the establishment and reactivation of latent infection in mice. *Journal of Virology* 61, 3528-3535 (1987)
18. K. Igarashi, R. Fawl, R. J. Roller, B. Roizman: Construction and properties of a recombinant herpes simplex virus 1 lacking both S-component origins of DNA synthesis. *Journal of Virology* 67, 2123-2132 (1993)
19. B. C. Herold, D. WuDunn, N. Soltys, P. G. Spear: Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. *Journal of Virology* 65, 1090-1098 (1991)
20. M. T. Shieh, D. WuDunn, R. I. Montgomery, J. D. Esko, P. G. Spear: Cell surface receptors for herpes simplex virus are heparan sulfate proteoglycans. *Journal of Cell Biology* 116, 1273-1281 (1992)
21. D. WuDunn, P. G. Spear: Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. *Journal of Virology* 63, 52-58 (1989)
22. P. G. Spear, R. J. Eisenberg, G. H. Cohen: Three classes of cell surface receptors for alphaherpesvirus entry. *Virology* 275, 1-8 (2000)
23. R. I. Montgomery, M. S. Warner, B. J. Lum, P. G. Spear: Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell* 87, 427-436 (1996)
24. J. C. Whitbeck, *et al.*: Glycoprotein D of herpes simplex virus (HSV) binds directly to HVEM, a member of the tumor necrosis factor receptor superfamily and a mediator of HSV entry. *Journal of Virology* 71, 6083-6093 (1997)
25. M. S. Warner, *et al.*: A cell surface protein with herpesvirus entry activity (HveB) confers susceptibility to infection by mutants of herpes simplex virus type 1, herpes simplex virus type 2 and pseudorabies virus. *Virology* 246, 179-189 (1998)
26. R. J. Geraghty, C. Krummenacher, G. H. Cohen, R. J. Eisenberg, P. G. Spear: Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus recptor. *Science* 280, 1618-1620 (1998)
27. F. Cocchi, *et al.*: The V domain of herpesvirus Ig-like receptor (HIgR) contains a major functional region in herpes simplex virus-1 entry into cells and interacts physically with the viral glycoprotein D. *Proceedings of the National Academy of Sciences, USA* 95, 15700-15705 (1998)
28. M. W. Ligas, D. C. Johnson: A herpes simplex virus mutant in which glycoprotein D sequences are replaced by beta-galactosidase sequences binds to but is unable to penetrate into cells. *Journal of Virology* 62, 1486-1494 (1988)
29. M. Sarmiento, M. Haffey, P. G. Spear: Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7(B2) in virion infectivity. *Journal of Virology* 29, 1149-1158 (1979)
30. A. Forrester, *et al.*: Construction and properties of a mutant of herpes simplex virus type 1 with glycoprotein H coding sequences deleted. *Journal of Virology* 66, 341-348 (1992)
31. B. Sodeik, M. W. Ebersold, A. Helenius: Microtubule-mediated transport of incoming herpes simplex virus 1 capsids to the nucleus. *Journal of Cell Biology* 136, 1007-1021 (1997)
32. W. Batterson, D. Furlong, B. Roizman: Molecular genetics of herpes simplex virus. VIII. Further characterization of a temperature-sensitive mutant defective in release of viral DNA and in other stages of the viral reproductive cycle. *Journal of Virology* 45, 397-407 (1983)
33. R. W. Honess, B. Roizman: Regulation of herpesvirus macro-molecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *Journal of Virology* 14, 8-19 (1974)
34. J. C. Alwine, W. L. Steinhart, C. W. Hill: Transcription of herpes simplex type 1 DNA in nuclei isolated from infected HEp-2 and KB cells. *Virology* 60, 302-307 (1974)
35. F. Costanzo, G. Campadelli-Fiume, L. Foa-Tomasi, E. Cassai: Evidence that herpes simplex virus DNA is transcribed by cellular RNA polymerase B. *Journal of Virology* 21, 996-1001 (1977)
36. W. Batterson, B. Roizman: Characterization of the herpes simplex virion-associated factor responsible for the induction of alpha genes. *Journal of Virology* 46, 371-377 (1983)
37. M. Katan, A. Haigh, C. P. Verrijzer, P. C. van der Vliet, P. O'Hare: Characterization of a cellular factor which interacts functionally with Oct-1 in the assembly of a multicomponent transcription complex. *Nucleic Acids Research* 18, 6871-6880 (1990)
38. T. M. Kristie, P. A. Sharp: Interactions of the Oct-1 POU subdomains with specific DNA sequences and with the HSV alpha-trans-activator protein. *Genes & Development* 4, 2383-2396 (1990)
39. B. T. Lin: Retinoblastoma cancer suppressor gene product is a substrate of the cell cycle regulator cdc2 kinase. *EMBO Journal* 10, 857-864 (1991)
40. S. Stern, W. Herr: The herpes simplex virus trans-activator VP16 recognizes the Oct-1 homeo domain: evidence for a homeo domain recognition subdomain. *Genes & Development* 5, 2555-2566 (1991)
41. K. F. Stringer, C. J. Ingles, J. Greenblatt: Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. *Nature* 345, 783-786 (1990)
42. P. Xiao, J. P. Capone: A cellular factor binds to the herpes simplex virus type 1 transactivator Vmw65 and is required for Vmw65-dependent protein-DNA complex assembly with Oct-1. *Molecular & Cellular Biology* 10, 4974-4977 (1990)
43. S. LaBoissiere, P. O'Hare: Analysis of HCF, the cellular cofactor of VP16, in herpes simplex virus-infected cells. *Journal of Virology* 74, 99-109 (2000)
44. I. H. Gelman, S. Silverstein: Identification of

immediate early genes from herpes simplex virus that transactivate the virus thymidine kinase gene. *Proceedings of the National Academy of Sciences, USA* 82, 5265-5269 (1985)
45. M. P. Quinlan, D. M. Knipe: Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral functions. *Molecular & Cellular Biology* 5, 957-963 (1985)
46. S. L. Sacks: The role of oral acyclovir in the management of genital herpes simplex. *Canadian Medical Association Journal* 136, 701-707 (1987)
47. N. D. Stow, E. C. Stow: Isolation and characterization of a herpes simplex virus type 1 mutant containing a deletion within the gene encoding the immediate early polypeptide Vmw110. *Journal of General Virology* 67, 2571-2585 (1986)
48. R. D. Everett: Construction and characterization of herpes simplex virus type 1 mutants with defined lesions in immediate early gene 1. *Journal of General Virology* 70, 1185-1202 (1989)
49. C. Van Sant, R. Hagglund, P. Lopez, B. Roizman: The infected cell protein 0 of herpes simplex virus 1 dynamically interacts with proteasomes, binds and activates the cdc34 E2 ubiquitin-conjugating enzyme, and possesses in vitro E3 ubiquitin ligase activity. *Proceedings of the National Academy of Sciences, USA* 98, 8815-8820 (2001)
50. G. G. Maul, R. D. Everett: The nuclear location of PML, a cellular member of the C3HC4 zinc-binding domain protein family, is rearranged during herpes simplex virus infection by the C3HC4 viral protein ICPO. *Journal of General Virology* 75, 1223-1233 (1994)
51. G. G. Maul, H. H. Guldner, J. G. Spivack: Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0). *Journal of General Virology* 74, 2679-2690 (1993)
52. R. A. Dixon, P. A. Schaffer: Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate early protein VP175. *Journal of Virology* 36, 189-203 (1980)
53. R. J. Watson, J. B. Clements: A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. *Nature* 285, 329-330 (1980)
54. S. W. Faber, K. W. Wilcox: Association of the herpes simplex virus regulatory protein ICP4 with specific nucleotide sequences in DNA. *Nucleic Acids Research* 14, 6067-6083 (1986)
55. T. M. Kristie, B. Roizman: Separation of sequences defining basal expression from those conferring alpha gene recognition within the regulatory domains of herpes simplex virus 1 alpha genes. *Proceedings of the National Academy of Sciences, USA* 81, 4065-4069 (1984)
56. S. Jean, K. M. LeVan, B. Song, M. Levine, D. M. Knipe: Herpes simplex virus 1 ICP27 is required for transcription of two viral late (gamma2) genes in infected cells. *Virology* 283, 273-284 (2001)
57. S. L. Uprichard, D. M. Knipe: Herpes simplex virus ICP27 mutant viruses exhibit reduced expression of specific DNA replication genes. *Journal of Virology* 70, 1969-1980 (1996)
58. W. R. Hardy, R. M. Sandri-Goldin: Herpes simplex virus inhibits host cell splicing, and regulatory protein ICP27 is required for this effect. *Journal of Virology* 68, 7790-7799 (1994)
59. A. Phelan, M. Carmo-Fonseca, J. McLaughlan, A. I. Lamond, J. B. Clements: A herpes simplex virus type 1 immediate-early gene product, IE63, regulates small nuclear ribonucleoprotein distribution. *Proceedings of the National Academy of Sciences, USA* 90, 9056-9060 (1993)
60. R. M. Sandri-Goldin, M. K. Hibbard, M. A. Hardwicke: The c-terminal repressor region of herpes simplex virus type 1 ICP27 is required for the redistribution of small nuclear ribonucleoprotein particles and splicing factor SC35; however, these alterations are not sufficient to inhibit host cell splicing. *Journal of Virology* 69, 6063-6076 (1995)
61. W. E. Mears, S. A. Rice: The herpes simplex virus immediate-early protein ICP27 shuttles bewteen nucleus and cytoplasm. *Virology* 242, 128-137 (1998)
62. T. M. Soliman, R. M. Sandri-Goldin, S. J. Silverstein: Shuttling of the herpes simplex virus type 1 regulatory protein ICP27 between the nucleus and cytoplasm mediates the expression of late proteins. *Journal of Virology* 71, 9188-9197 (1997)
63. M. C. Long, V. Leong, P. A. Schaffer, C. A. Spencer, S. A. Rice: ICP22 and the UL13 protein kinase are both required for herpes simplex virus-induced modification of the large subunit of RNA polymerase II. *Journal of Virology* 73, 5593-5604 (1999)
64. K. A. Carter, B. Roizman: The promoter and transcriptional unit of a novel herpes simplex virus 1 alpha gene are contained in, and encode a protein in frame with, the open reading frame of the alpha 22 gene. *Journal of Virology* 70, 172-178 (1996)
65. A. P. W. Poon, W. O. Ogle, B. Roizman: Posttranslational processing of infected cell protein 22 mediated by viral protein kinases is sensitive to amino acid substitution at distant sites and can be cell-type specific. *Journal of Virology* 71, 11210-11214 (2000)
66. A. G. Papavassiliou, S. J. Silverstein: Characterization of DNA-protein complex formation in nuclear extracts with a sequence from the herpes simplex virus thymidine kinase gene. *Journal of Biological Chemistry* 265, 1648-1657 (1990)
67. M. Carrozza, N. DeLuca: Interactions of the viral activator protein ICP4 with TFIID through TAF250. *Molecular and Cellular Biology* 16, 3085-3093 (1996)
68. C. A. Smith, P. Bates, R. Rivera-Gonzalez, B. Gu, N. A. DeLuca: ICP4, the major transcriptional regulatory protein of herpes simplex virus type 1, forms a tripartite complex with TATA-binding protein and TFIIB. *Journal of Virology* 67, 4676-4687 (1993)
69. F. McGregor, A. Phelan, J. Dunlop, J. B. Clements: Regulation of herpes simplex virus poly(A) site usage and the action of immediate-early protein IE63 in the early-late switch. *Journal of Virology* 70, 1931-1940 (1996)
70. L. A. Samaniego, A. L. Webb, N. A. DeLuca: Functional interactions between herpes simplex virus immediate early proteins during infection: Gene expression as a consequence of ICP27 and different domains of ICP4. *Journal of Virology* 69, 5705-5715 (1995)
71. M. Gao, D. M. Knipe: Potential role for herpes simplex virus ICP8 DNA replication protein in stimulation

of late gene expression. *Journal of Virology* 65, 2666-2675 (1991)
72. Y. M. Chen, D. M. Knipe: A dominant mutant form of the herpes simplex virus ICP8 protein decreases viral late gene transcription. *Virology* 221, 281-290 (1996)
73. E. E. McNamee, T. J. Taylor, D. M. Knipe: A dominant-negative herpesvirus protein inhibits intranuclear targeting of viral proteins: Effects on DNA replication and late gene expression. *Journal of Virology* 74, 10122-10131 (2000)
74. M. T. Muller: Binding of the herpes simplex virus immediate-early gene product ICP4 to its own transcription start site. *Journal of Virology* 61, 858-865 (1987)
75. R. Leopardi, N. Michael, B. Roizman: Repression of the herpes simplex virus 1 alpha 4 gene by its gene product (ICP4) within the context of the viral genome is conditioned by the distance and stereoaxial alignment of the ICP4 DNA binding site relative to the TATA box. *Journal of Virology* 69, 3042-3048 (1995)
76. P. J. Godowski, D. M. Knipe: Mutations in the major DNA-binding protein gene of herpes simplex virus type 1 result in increased levels of viral gene expression. *Journal of Virology* 47, 478-486 (1983)
77. P. J. Godowski, D. M. Knipe: Identification of a herpes simplex virus function that represses late gene expression from parental viral genomes. *Journal of Virology* 55, 357-365 (1985)
78. P. J. Godowski, D. M. Knipe: Transcriptional control of herpesvirus gene expression: gene functions required for positive and negative regulation. *Proceedings of the National Academy of Sciences, USA* 83, 256-260 (1986)
79. B. Roizman, M. Tognon: Restriction endonuclease patterns of herpes simplex virus DNA: application to diagnosis and molecular epidemiology. *Current Topics in Microbiology & Immunology* 104, 273-286 (1983)
80. A. A. Oroskar, G. S. Read: A mutant of herpes simplex virus type 1 exhibits increased stability of immediate early (alpha) mRNAs. *Journal of Virology* 61, 604-606 (1987)
81. B. D. Zelus, R. S. Stewart, J. Ross: The virion host shutoff protein of herpes simplex virus type 1: messenger ribonucleolytic activity in vitro. *Journal of Virology* 70, 2411-2419 (1996)
82. M. M. Elgadi, J. R. Smiley: Picornavirus internal ribosome entry site elements target RNA cleavage events induced by the herpes simplex virus virion host shutoff protein. *Journal of Virology* 73, 9222-9231 (1999)
83. B. M. Karr, G. S. Read: The virion host shutoff function of herpes simplex virus degrades the 5' end of a target mRNA before the 3' end. *Virology* 264, 195-204 (1999)
84. A. D. Kwong, J. A. Kruper, N. Frenkel: Herpes simplex virus virion host shutoff function. *Journal of Virology* 62, 912-921 (1988)
85. M. A. Hardwicke, R. M. Sandri-Goldin: The herpes simplex virus regulatory protein ICP27 contributes to the decrease in cellular mRNA levels during infection. *Journal of Virology* 68, 4797-4810 (1994)
86. E. K. Wagner, B. Roizman: Ribonucleic acid synthesis in cells infected with herpes simplex virus. I. Patterns of ribonucleic acid synthesis in productively infected cells. *Journal of Virology* 4, 36-46 (1969)
87. C. M. Preston, A. A. Newton: The effects of herpes simplex virus type 1 on cellular DNA-dependent RNA polymerase activities. *Journal of General Virology* 33, 471-482 (1976)
88. C. A. Spencer, M. E. Dahmus, S. A. Rice: Repression of host RNA polymerase II transcription by herpes simplex virus type 1. *Journal of Virology* 71, 2031-2040 (1997)
89. R. M. Sandri-Goldin, M. K. Hibbard: The herpes simplex virus type 1 regulatory protein ICP27 coimmunoprecipitates with anti-Sm antiserum, and the C terminus appears to be required for this interaction. *Journal of Virology* 70, 108-118 (1996)
90. R. D. Everett, W. C. Earnshaw, J. Findlay, P. Lomonte: Specific destruction of kinetochore protein CENP-C and disruption of cell division by herpes simplex virus immediate-early protein Vmw110. *EMBO Journal* 18, 1526-1538 (1999)
91. S. J. Advani, R. Brandimarti, R. R. Weichselbaum, B. Roizman: The disappearance of cyclins A and B and the increase in the acitvity of the G2/M phase cellular kinase cdc2 in herpes simplex virus 1-infected cells requires the expression of alpha 22/US1.5 and UL viral genes. *Journal of Virology* 74, 8-15 (2000)
92. S. J. Advani, R. R. Weichselbaum, B. Roizman: E2F proteins are postranslationally modified concomitantly with a reduction in nuclear binding activity in cells infected with herpes simplex virus 1. *Journal of Virology* 74, 7842-7850 (2000)
93. M. P. Quinlan, L. B. Chen, D. M. Knipe: The intranuclear location of a herpes simplex virus DNA-binding protein is determined by the status of viral DNA replication. *Cell* 36, 857-868 (1984)
94. A. M. Ishov, G. G. Maul: The periphery of nuclear domain 10 (ND10) as site of DNA virus deposition. *The Journal of Cell Biology* 134, 815-826 (1996)
95. R. J. Jacob, L. S. Morse, B. Roizman: Anatomy of herpes simplex virus DNA. XII. Accumulation of head-to-tail concatemers in nuclei of infected cells and their role in the generation of the four isomeric arrangements of viral DNA. *Journal of Virology* 29, 448-457 (1979)
96. M. D. Challberg: A method for identifying the viral genes required for herpesvirus DNA replication. *Proceedings of the National Academy of Sciences, USA* 83, 9094-9098 (1986)
97. P. Elias, M. E. O'Donnell, E. S. Mocarski, I. R. Lehman: A DNA binding protein specific for an origin of replication of herpes simplex virus type 1. *Proceedings of the National Academy of Sciences, USA* 83, 6322-6326 (1986)
98. A. J. Conley, D. M. Knipe, P. C. Jones, B. Roizman: Molecular genetics of herpes simplex virus. VII. Characterization of a temperature-sensitive mutant produced by in vitro mutagenesis and defective in DNA synthesis and accumulation of gamma polypeptides. *Journal of Virology* 37, 191-206 (1981)
99. D. J. Purifoy, R. B. Lewis, K. L. Powell: Identification of herpes simplex virus DNA polymerase gene. *Nature* 269, 621-23 (1977)
100. P. Elias, I. R. Lehman: Interaction of origin binding protein with an origin of replication of herpes simplex virus 1. *Proceedings of the National Academy of Sciences, USA* 85, 2959-2963 (1988)

101. A. Koff, P. Tegtmeyer: Characterization of major recognition sequences for a herpes simplex virus type 1 origin-binding protein. *Journal of Virology* 62, 4096-4103 (1988)
102. W. T. Ruyechan: The major herpes simplex virus DNA-binding protein holds single-stranded DNA in an extended configuration. *Journal of Virology* 46, 661-666 (1983)
103. C. K. Lee, D. M. Knipe: An immunoassay for the study of DNA-binding activities of herpes simplex virus protein ICP8. *Journal of Virology* 54, 731-738 (1985)
104. R. E. Dutch, I. R. Lehman: Renaturation of complementary DNA strands by herpes simplex virus type 1 ICP8. *Journal of Virology* 67, 6945-6949 (1993)
105. P. E. Boehmer, M. S. Dodson, I. R. Lehman: The herpes simplex virus type-1 origin binding protein. DNA helicase activity. *Journal of Biological Chemistry* 268, 1220-1225 (1993)
106. M. Falkenberg, D. A. Bushnell, P. Elias, I. R. Lehman: The UL8 subunit of the heterotrimeric herpes simplex virus type 1 helicase-primase is required for the unwinding of single strand DNA-binding protein (ICP8)-coated DNA substrates. *Journal of Biol Chem* 272, 22766-22770 (1997)
107. N. Tanguy LeGac, G. Villani, P. E. Boehmer: Herpes simplex virus type-1 single-stranded DNA-binding protein (ICP8) enhances the ability of the viral DNA helicase-primase to unwind cisplatin-modified DNA. *Journal of Biological Chemistry* 273, 13801-13807 (1998)
108. H. C. Chiou, S. K. Weller, D. M. Coen: Mutations in the herpes simplex virus major DNA-binding protein gene leading to altered sensitivity to DNA polymerase inhibitors. *Virology* 145, 213-226 (1985)
109. A. de Bruyn Kops, D. M. Knipe: Formation of DNA replication structures in herpes virus-infected cells requires a viral DNA binding protein. *Cell* 55, 857-868 (1988)
110. M. Bush, *et al.*: Correct intranuclear localization of herpes simplex virus DNA polymerase requires the viral ICP8 DNA-binding protein. *Journal of Virology* 65, 1082-1089 (1991)
111. J. J. Crute, E. S. Mocarski, I. R. Lehman: A DNA helicase induced by herpes simplex virus type 1. *Nucleic Acids Research* 16, 6585-6596 (1988)
112. H. M. Keir, E. Gold: Deoxyribonucleotidyl transferase and deoxyribonuclease from cultured cells infected with herpes simplex virus. *Biochemistry & Biophysics Acta Review* 72, 263-276 (1963)
113. J. S. Gibbs, H. C. Chiou, K. F. Bastow, Y. C. Cheng, D. M. Coen: Identification of amino acids in herpes simplex virus DNA polymerase involved in substrate and drug recognition. *Proceedings of the National Academy of Sciences, USA* 85, 6672-6676 (1988)
114. S. Kit, D. R. Dubbs: Properties of deoxythymidine kinase partially purified from noninfected and virus-infected mouse fibroblast cells. *Virology* 26, 16-27 (1965)
115. S. Bacchetti, M. J. Evelegh, B. Muirhead: Identification and separation of the two subunits of the herpes simplex virus ribonucleotide reductase. *Journal of Virology* 57, 1177-1181 (1986)
116. S. Caradonna, D. Worrad, R. Lirette: Isolation of a herpes simplex virus cDNA encoding the DNA repair enzyme uracil-DNA glycosylase. *Journal of Virology* 61, 3040-3047 (1987)
117. J. A. Fyfe, P. M. Keller, P. A. Furman, R. L. Miller, G. B. Elion: Thymidine kinase from herpes simplex virus phosphorylates the new antiviral compound, 9-(2-hydroxyethoxymethyl)guanine. *Journal of Biological Chemistry* 253, 8721-8727 (1978)
118. W. W. Newcomb, *et al.*: Structure of the herpes simplex virus capsid. Molecular composition of the pentons and the triplexes. *Journal of Molecular Biology* 232, 499-511 (1993)
119. W. W. Newcomb, *et al.*: Assembly of the herpes simplex virus capsid: Characterization of intermediates observed during cell-free capsid formation. *Journal of Molecular Biology* 263, 432-446 (1996)
120. W. W. Newcomb, *et al.*: Assembly of the herpes simplex virus procapsid from purified components and identification of small complexes containing the major capsid and scaffolding proteins. *Journal of Virology* 73, 4239-4250 (1999)
121. B. F. Ladin, M. L. Blankenship, T. Ben-Porat: Replication of herpesvirus DNA II. The maturation of concatemeric DNA of pseudo rabies virus to genome length is related to capsid formation. *Journal of Virology* 33, 1151-1164 (1980)
122. B. F. Ladin, S. Ihara, H. Hampl, T. Ben-Porat: Pathway of assembly of herpesvirus capsids: An analysis using DNA temperature sensitive mutants of pseudorabies virus. *Virology* 116, 544-561 (1982)
123. S. L. Varmuza, J. R. Smiley: Signals for site-specific cleavage of HSV DNA: maturation involves two separate cleavage events at sites distal to the recognition sequences. *Cell* 41, 793-802 (1985)
124. L. P. Deiss, J. Chou, N. Frenkel: Functional domains within the a sequence involved in the cleavage-packaging of herpes simplex virus DNA. *Journal of Virology* 59, 605-618 (1986)
125. D. A. Vlazny, A. Kwong, N. Frenkel: Site-specific cleavage/packaging of herpes simplex virus DNA and the selective maturation of nucleocapsids containing full-length viral DNA. *Proceedings of the National Academy of Sciences, USA* 79, 1423-1427 (1982)
126. P. Siminoff, M. G. Menefee: Normal and 5-bromodeoxyuridine-inhibited development of herpes simplex virus. An electron microscope study. *Experimental Cell Research* 44, 241-255 (1966)
127. C. W. Stackpole: Herpes-type virus of the frog renal adenocarcinoma. I. Virus development in tumor transplants maintained at low temperature. *Journal of Virology* 4, 75-93 (1969)
128. L. W. Enquist, P. J. Husak, B. W. Banfield, G. A. Smith: Infection and spread of alphaherpesviruses in the nervous system. *Advances Virus Research* 51, 237-247 (1999)
129. J. N. Skepper, A. Whiteley, H. Browne, A. Minson: Herpes simplex virus nucleocapsids mature to progeny virions by an envelopment ' deenvelopment ' reenvelopment pathway. *Journal of Virology* 75, 5697-5702 (2001)
130. H. Granzow, *et al.*: Egress of alphaherpesviruses: Comparative ultrastructural study. *Journal of Virology* 75, 3675-3684 (2001)
131. D. L. Rock, N. W. Fraser: Latent herpes simplex

virus type 1 DNA contains two copies of the virion DNA joint region. *Journal of Virology* 55, 849-852 (1985)
132. D. M. Mellerick, N. W. Fraser: Physical state of the latent herpes simplex virus genome in a mouse model system: evidence suggesting an episomal state. *Virology* 158, 265-275 (1987)
133. C. A. Carton, E. D. Kilbourne: Activation of latent herpes simplex by trigeminal sensory-root section. *New England Journal of Medicine* 246, 172-176 (1952)
134. J. G. Stevens, E. K. Wagner, G. B. Devi-Rao, M. L. Cook, L. T. Feldman: RNA complementary to a herpesvirus alpha gene mRNA is prominent in latently infected neurons. *Science* 235, 1056-1059 (1987)
135. D. L. Rock, *et al.*: Detection of latency-related viral RNAs in trigeminal ganglia of rabbits latently infected with herpes simplex virus type 1. *Journal of Virology* 61, 3820-3826 (1987)
136. P. R. Krause, K. D. Croen, S. E. Straus, J. M. Ostrove: Detection and preliminary characterization of herpes simplex virus type 1 transcripts in latently infected human trigeminal ganglia. *Journal of Virology* 62, 4819-4823 (1988)
137. D. A. Garber, P. A. Schaffer, D. M. Knipe: A LAT-associated function reduces productive-cycle gene expression during acute infection of murine sensory neurons with herpes simplex virus type 1. *Journal of Virology* 71, 5885-5893 (1997)
138. S. H. Chen, M. F. Kramer, P. A. Schaffer, D. M. Coen: A viral function represses accumulation of transcripts from productive-cycle genes in mouse ganglia latently infected with herpes simplex virus. *Journal of Virology* 71, 5878-84 (1997)
139. G.-C. Perng, *et al.*: Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript. *Science* 287, 1500-1503 (2000)

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Send correspondence to: Dr David M Knipe, Harvard Medical School, Department of Microbiology and Molecular Genetics, 200 Longwood Avenue, Boston, MA 02115, Tel: 617-432-1934, Fax:: 617-432-0223, E-mail: david_knipe@hms.harvard.edu