Chapter 9

Herpesviruses

Chapter Outline

We are familiar with *herpesviruses*,[1] because most of us have suffered their infection. Herpesviruses are associated with multiple human disorders, ranging from mild symptoms such as cold sores to more severe diseases, such as cancers (Table 9.1). For instance, oral herpes is caused by herpes simplex virus type 1 (HSV-1) (Fig. 9.1). Here, HSV-1, a prototype of human alpha-herpesvirus, will be mainly described. Besides HSV-1, human cytomegalovirus (HCMV), a prototype of beta-herpesvirus, and Epstein-Barr virus (EBV), a prototype of gamma-herpesvirus, will be described briefly in separate boxes.

9.1 CLASSIFICATION

Taxonomy: Herpesviruses are found not only in human but also in many vertebrates such as mammals, birds, reptiles, and fishes. Over 130 species of herpesviruses have been reported, and they can be divided into three subgroups depending on their biological properties: alpha-, beta-, and gamma-herpesviruses.

Eight species of human herpesviruses have been discovered. Human herpesviruses are divided into three genera: alpha-, beta-, and gamma-herpesviruses (Table 9.2). In alpha-herpesviruses, three species are reported: (1) herpes simplex virus type 1 (HSV-1), which causes cold sores or oral herpes, (2) herpes simplex virus type 2 (HSV-2), which causes genital herpes, and (3) Varicella-Zoster virus (VZV), which causes chicken pox in children and shingles in adults. Beta-herpesviruses include human cytomegalovirus (HCMV), which causes cytomegalic inclusion disease, and human herpesvirus 6 and 7, which cause roseola. In gamma-herpesviruses, two species are reported: *Epstein-Barr virus (EBV)*[2] and Kaposi's sarcoma-associated herpesvirus (*KSHV*). EBV is associated with two types of human cancers: Burkitt's lymphoma and nasopharyngeal carcinoma. KSHV is associated with Kaposi's sarcoma in AIDS patients. These human gamma-herpesviruses are *tumor viruses*.[3] In addition, human herpesviruses are systemically named by the order of discovery, from HHV-1 to HHV-8 (see Table 9.2).

Of the eight human herpesviruses, HSV-1, a prototype of human alpha-herpesvirus, will be mainly described for brevity.

1. **Herpesvirus** the term "herpes" is derived from Greek word for "creep = latent or chronic"-*herpin*.

2. **Epstein-Barr virus (EBV)** A human gamma-herpesvirus that is associated with Burkitt's lymphoma. The virus was named after two scientists who discovered: Dr. Epstein and Dr. Barr (see Box 9.3).

3. **Tumor virus** Five human viruses, including EBV and KSHV, are known to be an etiologic agent for human cancer (see Table 24.2).

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TABLE 9.1 The Defining Features of Herpesvirus

Genome	Particle Structure	Replication Mechanism
Large linear dsDNA (120 ~ 235 kb)	Enveloped	Viral DNA polymerase
Terminal repeats	Icosahedral nucleocapsid	Rolling-circle mechanism
Life Cycle	**Host Effect**	**Disease**
IE-E-L phase	Host shutoff	Cold sore, chicken pox, zoster
Latent infection	Immune evasion	Tumor (lymphoma)

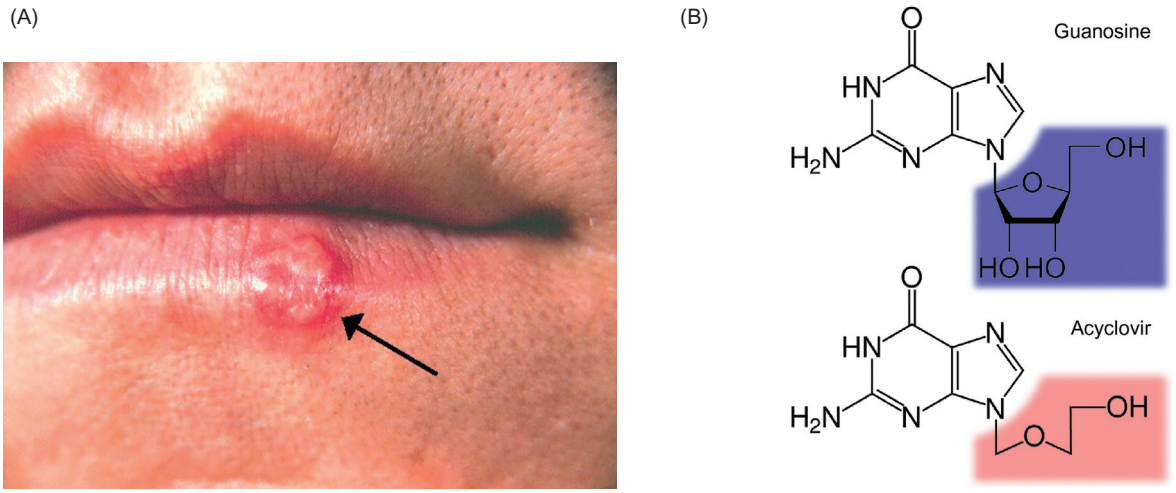

FIGURE 9.1 Oral herpes and antiherpes drugs. (A) Herpes simplex lesion of lower lip, second day after onset. Oral herpes, the visible symptoms of which are colloquially called *cold sores* or *fever blisters*, is the most common form of herpesvirus infection. (B) Comparison of chemical structures of guanosine and guanosine analog acyclovir. Acyclovir is an antiherpes drug prescribed for cold sores.

9.2 THE VIRION AND GENOME STRUCTURE OF HSV-1

Virion Structure: HSV-1 is an enveloped virus with a diameter of 150 nm, in which the capsid with a diameter of 90 nm is encompassed (Fig. 9.2). In the viral envelope, more than nine viral envelope glycoproteins, ranging from gB to gN glycoprotein, are embedded. A peculiar feature of the virion structure of herpesviruses is that the space between the envelope and the capsid, termed *tegument*,[4] is filled with numerous viral proteins. Importantly, the tegument proteins are essential for the establishment of virus infection. Among more than 15 viral tegument proteins identified, three of them are well characterized: VP16, ICP0, and Vhs protein. *VP16* is a tegument protein that acts as a transcriptional transactivator of the viral genes (ie, immediate early genes). *ICP0*, the viral ubiquitin E3 ligase, targets a number of cellular proteins that restrict viral infection for the degradation (see Fig. 9.7). On the other hand, *Vhs*[5] protein counteracts host innate immune response by reducing mRNA stability, in particular, those of interferons and pro-inflammatory cytokines.

Genome Structure: HSV-1 has a large 150 kb linear double-strand DNA (Fig. 9.3). Two kinds of repeat elements are present in the genome: (1) a pair of direct repeat elements at both termini, termed *TR* (terminal repeat), and (2) two pairs of inverted repeat elements in the middle of the genome, termed *IR* (inverted repeat). TR is essential for viral DNA genome packaging. However, the biological function of IR remains uncertain. Obviously, the vast majority of

4. **Tegument** A space between envelope and nucleocapsid in virions of certain viruses, notably herpesviruses. Tegument proteins can be compared with matrix protein in other enveloped virions.

5. **Vhs (virion host shutoff)** It blocks host immune response by degradation of cellular mRNAs via its endonuclease activity.

TABLE 9.2 Classification of Human Herpesviruses

Human Herpesviruses	Common Name	Disease
Alpha-Herpesvirus		
Herpes simplex virus type-1	HSV-1 (HHV-1)	Cold sore
Herpes simplex virus type-2	HSV-2 (HHV-2)	Genital ulcer
Varicella-Zoster virus	VZV (HHV-3)	Chicken pox or varicella, zoster
Beta-Herpesvirus		
Human cytomegalovirus	HCMV (HHV-5)	Mononucleosis
		Cytomegalic inclusion disease
Human herpesvirus 6	HHV-6	Roseola
Human herpesvirus 7	HHV-7	Roseola
Gamma-Herpesvirus		
Epstein-Barr virus	EBV (HHV-4)	Infectious mononucleosis
		Burkitt's lymphoma
		Nasopharyngeal carcinoma
Kapposi's sarcoma	KSHV (HHV-8)	Karposi's sarcoma

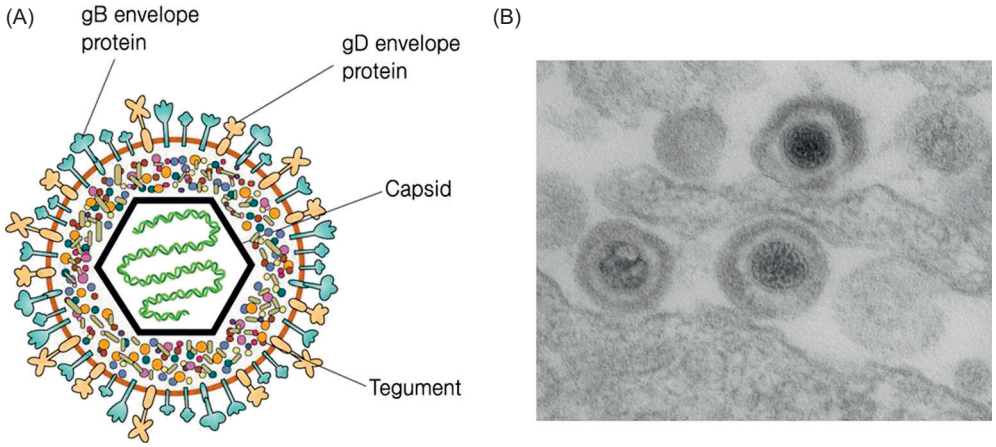

FIGURE 9.2 **Virion structure of HSV-1.** (A) Multiple envelope glycoproteins, including gB and gD, are present in the viral envelope. Tegument is stuffed with numerous viral proteins such as VP16 and Vhs. The viral capsid is larger, with $T = 16$ symmetry, which packages a linear double-strand DNA with 150 kb in length. (B) Electron micrograph of HSV-1 virion. Tegument, a space between the capsid and the envelope, is clearly visible.

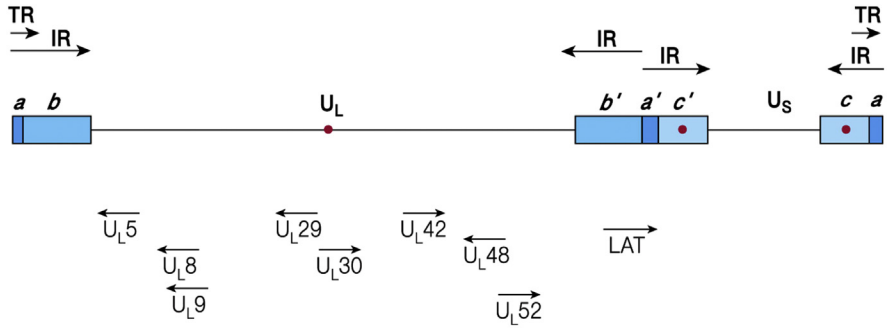

FIGURE 9.3 **Genome organization of HSV-1.** The linear double strand DNA genome of HSV-1 is drawn with emphasis on repeat elements (box), which are denoted by "a," "b," and "c." U_L and U_S elements are demarcated by two neighboring IR elements ("ab" or "ac," respectively). Note that "**a**′" is complement of "**a**." The terminal repeats ("a") at the both ends of the genome are denoted by TR, while the inverted repeats are denoted by IR. Arrows indicate the directionality of the nucleotide sequence. OriL located in U_L region and OriS located in "c" elements are indicated by red dots. A subset of HSV-1 transcripts is also indicated below. *LAT*, latency-associated transcript.

DNA genome sequence is located between two IRs, and these are aptly named U_L (unique long) and U_S (unique short), as opposed to the "repeat" element, which is reserved for a redundant element. Most of the viral proteins are encoded within U_L and U_S regions. Intriguingly, three origins of DNA replication (Ori) elements are found: one in U_L region (termed *OriL*) and two in U_S region (termed *OriS*).

P*rotein Coding*: Having a large genome, HSV-1 encodes over 90 proteins (see Fig. 9.3). The U_L region encodes 65 proteins, while the U_S region encodes 14 proteins. Interestingly, a few viral proteins are encoded even by the repeat elements. The viral proteins are named after their gene number. For instance, U_L 30 refers to the gene number 30 that is located in the U_L region; the protein encoded by the U_L 30 gene is termed U_L 30 protein.

Multiple nomenclatures of the viral proteins were used in the past. Recently, the nomenclature according to the gene number became widely accepted, as this is systemic and less confusing. For instance, VP16 is called by three different nomenclatures: (1) *VP16* is named after structural proteins constituting virion particle, (2) *UL48* is named after the gene number, and (3) *ICP25*[6] is named after the serial number of viral proteins expressed in the infected cells. For a given protein, one of these is more frequently used than others; in fact, VP16 is more commonly used than U_L48 and ICP25.

9.3 THE LIFE CYCLE OF HSV-1

Cell tropism: HSV-1 infects the *epithelium* of the mucosal layer. Following primary infection, HSV-1 invades the central nerve system (CNS) via sensory neurons and finally establishes latent infection in nerve ganglia (see Fig. 9.12).

Similar to other DNA viruses, the virus life cycle can be divided into two phases by the onset of viral genome replication: early phase and late phase. The viral genome replication occurs in the nucleus of infected cells (Fig. 9.4).

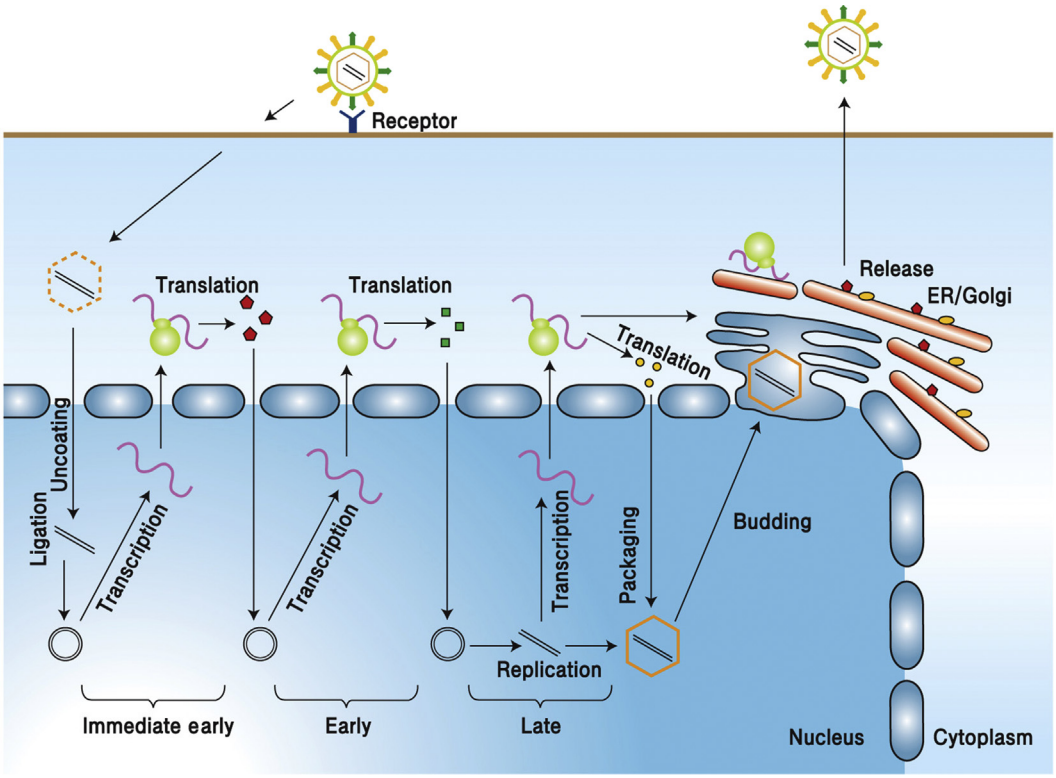

FIGURE 9.4 The life cycle of HSV-1. The virion enters the cell by either direct fusion or endocytosis (for clarity, only entry by direct fusion is shown). Following entry to cell, the linear DNA genome is delivered to the nucleus, and converted into a circular form in the nucleus. The viral gene expression can be divided into three phases: immediate early phase, early phase, and late phase. The capsid assembly occurs in the nucleus. The assembled capsid egresses via nuclear membrane, and then via secretory pathway.

6. **ICP** (infected cell protein)

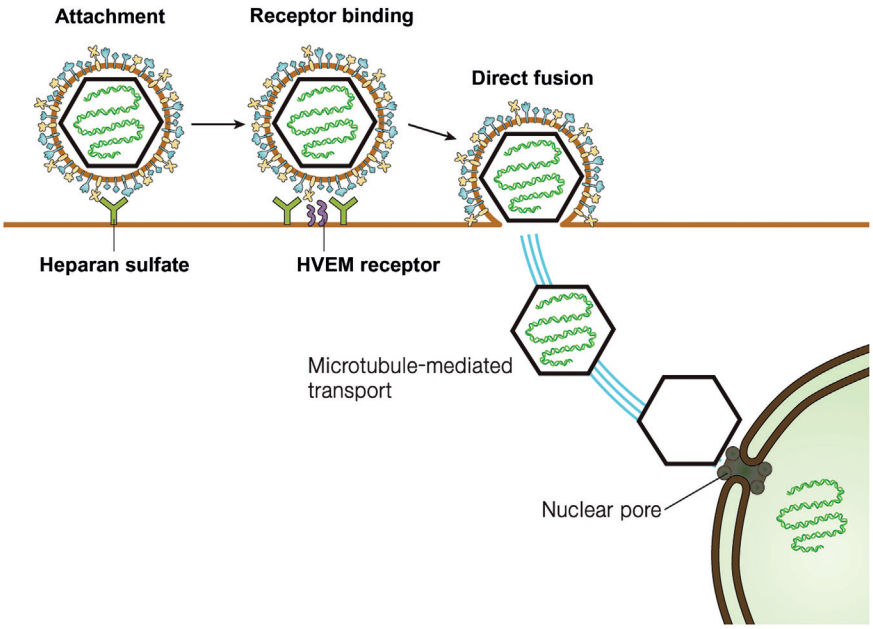

FIGURE 9.5 The HSV-1 entry via direct fusion. The virion attaches to cell surface via heparan sulfate as attachment factors. The gD binds to an entry receptor, HVEM. This gD-HVEM interaction triggers gB, the fusion protein, to mediate membrane fusion. Following the penetration, the viral capsid trafficks toward the nucleus via microtubule-mediated transport. Upon docking to the nuclear pore, the capsids become partially disrupted, and then release their genomes into the nucleus.

Entry: HSV-1 enters cells either via direct fusion or receptor-mediated endocytosis. Virion attaches to cell surface glycosaminoglycans such as heparan sulfate for entry (Fig. 9.5). Several viral glycoproteins, such as gD and gB, are involved in membrane fusion with the host cell. In particular, gD interacts with a cellular receptor, *HVEM*.[7] This subsequently triggers gB, the fusion protein, to mediate membrane fusion. Following penetration to the cytoplasm, the capsids traffic to nuclear membrane via *microtubule-mediated transport*. Upon docking to the nuclear pore, the viral DNA released from the capsids enters the nucleus via a nuclear pore. The linear DNA genome becomes converted to a circular configuration. The circularization of DNA genome is facilitated by ligation of "**a**" elements at the end or by recombination between "**a**" elements (see Fig. 9.3).

9.3.1 Viral Gene Expression

Upon entry to the nucleus, the viral DNA is localized near nuclear bodies known as *nuclear domain 10*[8] (ND10), where the viral chromosome exists in a repressed state. In other words, ND10 represents the host *intrinsic immunity* against the viral invasion. The viral gene transcription takes place only after the ICP0-mediated epigenetic regulation.

Three Phases: Prior to viral genome replication, the viral transcription takes place, and these genes are termed "early genes." Like adenovirus, early gene transcription of herpesvirus is subdivided into two phases: these are termed "immediate early genes (IE)," and "early genes (E)." Immediate early gene expression is stimulated by viral tegument proteins, while early gene expression is stimulated by immediate early gene products (Fig. 9.6). Early gene products are largely involved in the viral genome replication, and trigger the switch from early to late phase. In summary, the viral genes are divided into three groups, depending on the phase of the life cycle; immediate early (IE), early (E), and late (L) genes. Alternatively, they are also called α, β, and γ genes, respectively.

Immediate Early Phase: Prior to the onset of viral genome replication, immediate early and early gene expression occurs in a sequential manner. *VP16*, a tegument protein, acts as the transcriptional transactivator of IE (immediate early) genes. It binds to the promoter region of IE genes and potently transactivates transcription of IE genes. In addition, *Vhs*, a tegument protein, acts to degrade cellular mRNAs for few hours after infection. Because of Vhs function, the viral mRNAs are preferentially accumulated in the cytoplasm, as opposed to host mRNAs. Interestingly, Vhs was shown to degrade even the immediate early mRNA, which is no longer needed, during early phase. In late phase, on the other hand, the nascent made VP16 blocks the RNase activity of Vhs via direct binding.

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7. **HVEM (herpes virus entry mediator)** It is a membrane protein that belongs to TNF-α superfamily.

8. **Nuclear domain 10 (ND10)** A small proteineous subnuclear structure that is composed of multiple factors including PML (promyelocytic leukemia protein) and Sp100. It is also called PML nuclear bodies.

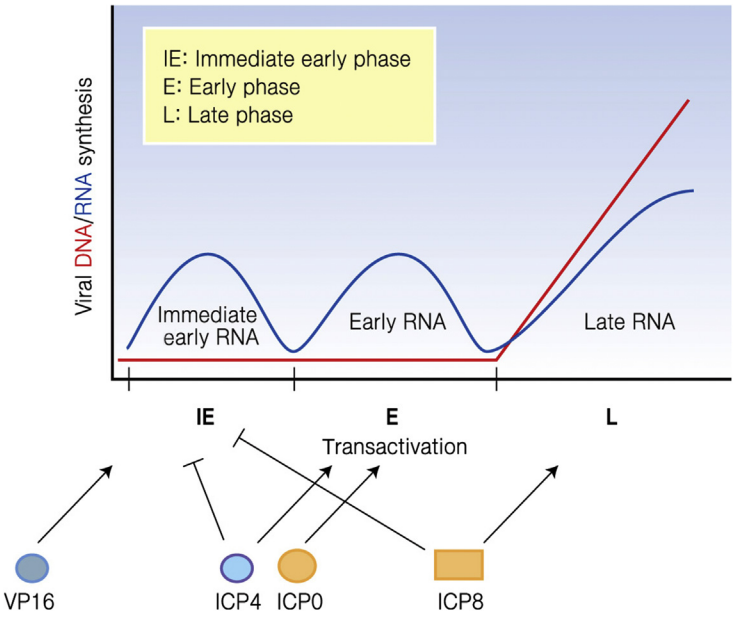


FIGURE 9.6 A graphic illustrating the switch from early to late phase. VP16 triggers transcription of immediate early genes (IE). ICP4 and ICP0 trigger the switch from immediate early (IE) to early (E) phase, while ICP8 triggers the switch from early to late (L) phase. The level of viral RNA and viral DNA are drawn in arbitrary unit for the purpose of explanation.

TABLE 9.3 The Immediate Early Phase Proteins of HSV-1 and Their Functions

Protein	Function
ICP0	Transcriptional transactivator and the viral ubiquitin E3 ligase
ICP4	Transcriptional transactivator
ICP27	Blocks cellular RNA splicing; facilitates viral mRNA export
ICP47	Blocks antigen presentation by binding to TAP transporter

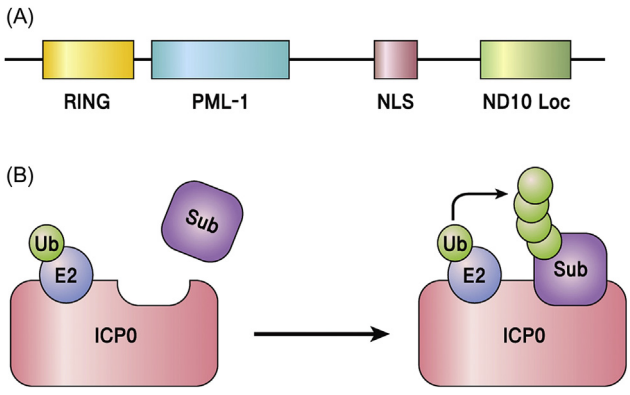


FIGURE 9.7 ICP0 protein: functional domains. (A) The functional domain of ICP0 protein, including RING finger, PML-1 binding region, NLS sequence, and ND10 localization domain. (B) A diagram illustrating substrate-targeting mechanism employed by ICP0 ubiquitin E3 ligase.

Switch to Early Phase: The functions of some IE gene products are well known (Table 9.3). Two IE gene products, *ICP0* and *ICP4*, play a role in switching from immediate early to early phase via transactivation of early gene transcription (see Fig. 9.6). ICP4 is also required for late gene transcription. ICP0 is a multifunctional protein, which interacts with numerous cellular factors including PML (Fig. 9.7). Moreover, ICP0 has a RING finger domain that confers *ubiquitin E3 ligase activity*. ICP0 influences chromatin structure in that it impedes the assembly of a repressed viral

chromatin structures. As stated above, ICP0-mediated inactivation of host intrinsic restriction factors is essential for viral transcription. Whether or not ICP0 influences chromatic structure directly through its ubiquitin E3 ligase activity remains unclear. In contrast, ICP4 enhances transcription via recruiting cellular transcription factors to the promoter regions. Conversely, ICP4 can repress transcription of its own gene by directly binding to the promoter region (ie, "auto-regulation").

On the other hand, *ICP27* blocks the nuclear export of cellular mRNAs, but nonetheless facilitates the nuclear export of the viral mRNAs. In other word, ICP27 promotes viral gene expression by regulating mRNA processing (see Table 9.3). Only 4 h after viral infection, IE gene expression begins to decrease. The downregulation of IE gene expression occurs primarily by Vhs and by ICP4, which suppresses IE gene transcription (see Fig. 9.6).

Early Phase: Early gene products are mainly involved in viral genome replication (see Table 9.4). In particular, *ICP8* triggers the switch from early to late phase, by suppressing IE gene expression as well as by promoting late gene expression (see Fig. 9.6).

9.3.2 Late Phase

The viral genome replication, the late gene expression, the capsid assembly, and the release of the assembled capsid occur in the late phase.

Genome Replication: Factors essential for viral DNA synthesis can be divided into two groups: *cis*-acting elements and *trans*-acting factors. First, the origin of replication, the site for initiation of DNA synthesis, represents *cis*-acting elements for DNA synthesis. HSV-1 genome contains three origin of replication (one *OriL* and two *OriS*); these three elements are functionally redundant, as only one of them is sufficient for viral DNA replication.

Regarding *trans*-acting factors, *UL30* serves as a viral DNA polymerase for the HSV-1 genome replication (Fig. 9.8). Besides, six additional viral proteins contribute to the viral genome replication (Table 9.4). These

TABLE 9.4 The DNA Replication Proteins of HSV-1

Protein	Function
U_L9	Origin-binding protein; ATPase & helicase activity
U_L9 (ICP8)	Single-strand DNA-binding protein
$U_L5/U_L8/U_L52$	Helicase-primase complex
U_L30	DNA polymerase
U_L42	Processivity factor

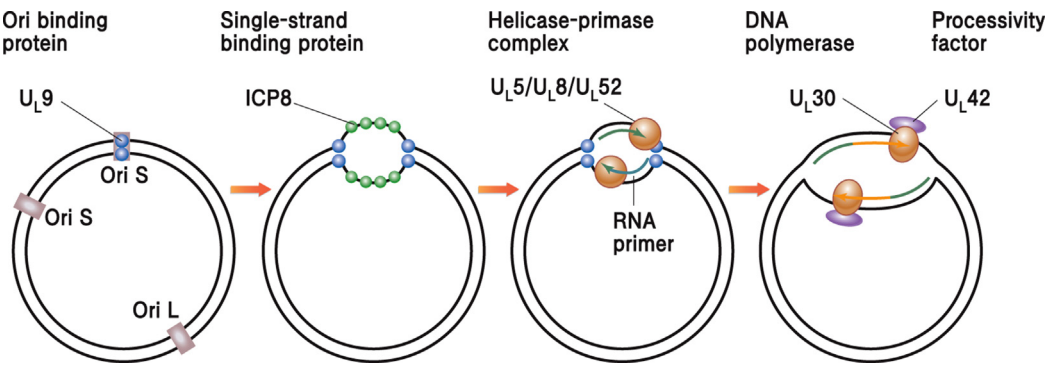


FIGURE 9.8 The θ form DNA replication. The initiation of DNA synthesis at OriS is shown as an example out of three origins. U_L9 binds to OriS to unwind the duplex DNA. Then, ICP8 binds to single-strand DNA region, recruiting $U_L5/U_L8/U_L52$ helicase-primase complex to synthesize RNA primer. Finally, U_L30, the viral DNA polymerase, continues to synthesize the DNA from the RNA primer. U_L42 acts as a processivity factor. This mode of DNA replication is also called "bidirectional DNA replication," as the nascent DNA synthesis occurs simultaneously in both strands in opposite directions.

include: (1) *UL9*, the origin-binding protein, (2) *UL5/UL8/UL52* complex, helicase-primase complex, and (3) *UL48*, a processivity factor. Moreover, *ICP8* (U_L48) is a single strand DNA-binding protein, which stimulates viral DNA synthesis.

The mechanism for HSV-1 genome replication is not fully understood. The reason for this is primarily due to the lack of an in vitro system that recapitulates viral DNA synthesis. Nonetheless, the mechanism of the viral DNA synthesis is expected to be fundamentally similar with that of the cellular genome. The circularized DNA genome, which is formed immediately after its entry into the nucleus, is the template for the viral genome replication. First, the DNA replication begins via bidirectional DNA replication by using the circular template (see Fig. 9.8). This mode of DNA replication is dubbed "*θ form replication*"; it was named after the shape of the DNA intermediates. Nonetheless, the bulk of DNA synthesis is achieved by a so-called *rolling-circle mechanism* (Fig. 9.9). The evidence for this is the *concatemers*, the multimeric form of the DNA genome, found during the viral genome replication. It is believed that viral genome replication starts with the θ form replication, and then, the mode of DNA replication switches from *θ* form replication to the rolling-circle mechanism. It is thought that the nick is made in one strand of DNA prior to the switch.

What is the biological significance of the circularization of linear DNA prior to the genome replication? It is believed that the circularization of linear DNA is the viral strategy to overcome "*the end-replication problem*," the problems inherent to the linear DNA genome (see Box 10.2).

Having a larger genome, herpesviruses encode genes pertaining to nucleic acids metabolism as well. Many of these viral proteins contribute to the viral DNA synthesis. For instance, thymidine kinase (*UL23*), ribonucleotide reductase (*UL39/UL40*), and deoxyuridine triphosphatase (*UL50*) are involved in dNTPs synthesis. *N*-glycosylase (*UL2*) is involved in DNA repair. Interestingly, these metabolic enzymes are dispensable for dividing cells, which carry out DNA synthesis themselves, but indispensable for nondividing cells such as neuronal cells.

Late Gene Expression: Once viral DNA replication has initiated, viral late gene expression is increased. Viral immediate early proteins, such as ICP4, ICP22, and ICP27, and early gene products, such as ICP8, are required for late gene expression (see Fig. 9.6). Late gene transcription and DNA replication as well as virion assembly take place in *replication compartments* in the nucleus.

Assembly and Release: The capsid assembly occurs in the nucleus. Unlike most other DNA viruses, the capsids are preassembled first, and then one unit of viral genome DNA is subsequently packaged into the preassembled capsid (Fig. 9.10). The sequence element termed "**a**" element serves as a *packaging signal*. When one unit of the viral genome, from "a" to "a," is enclosed into the capsid, the DNA is cleaved by the cleavage and packaging proteins. In other words, the DNA genome packaging is completed by the cleavage of the concatemer. The viral factors involved in the cleavage and packaging remains to be identified.

Cell lysis is the way to release the assembled capsid for most DNA viruses that replicate in the nucleus. The DNA viruses we have seen so far are all nonenveloped viruses, including polyomavirus, papillomavirus, and adenovirus. In contrast, being an enveloped virus, herpesviruses need to acquire the envelope. In particular, herpesviruses acquire their envelope from the nuclear membrane in the first place (Fig. 9.11). In fact, herpesviruses bud into the nuclear envelope (*primary envelopment*) before entering the cytoplasm; as a matter of fact, herpesviruses are the only known viruses that bud into the nuclear envelope. The evidence for this exit is that enveloped virions residing inside perinuclear space are observed by electron microscopy. Once the virion resides inside the perinuclear space, the membrane fusion of the virion with the outer nuclear membrane, termed "*de-envelopment*," releases the naked capsid into the cytosol. After all, the viral capsids transit the two nuclear membranes; this peculiar nuclear exit process is dubbed "*nuclear egress*." Once located in the cytoplasm, the viral capsid acquires the envelope again from

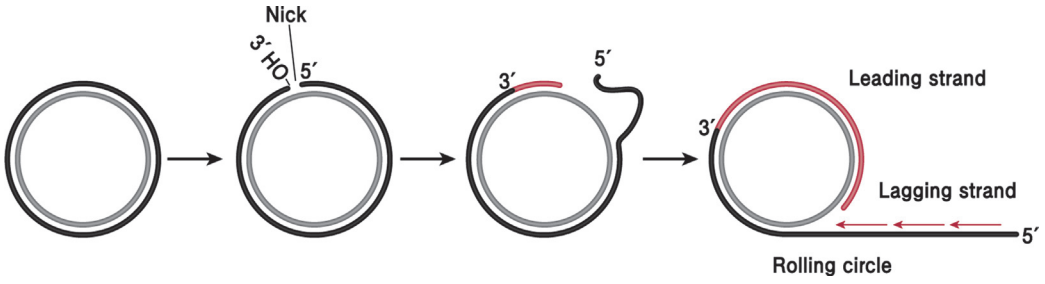


FIGURE 9.9 The rolling-circle replication mechanism of HSV-1 DNA replication. The nick generated in one strand of circular DNA provides 3′ hydroxyl group for the DNA synthesis. The DNA synthesis by using the circular template constitutes the leading strand. The continued DNA synthesis displaces the nicked strand, and it becomes a template for the discontinued DNA synthesis, constituting the lagging strand. As a result of rolling-circle replication, multiple genome length concatemers are synthesized.

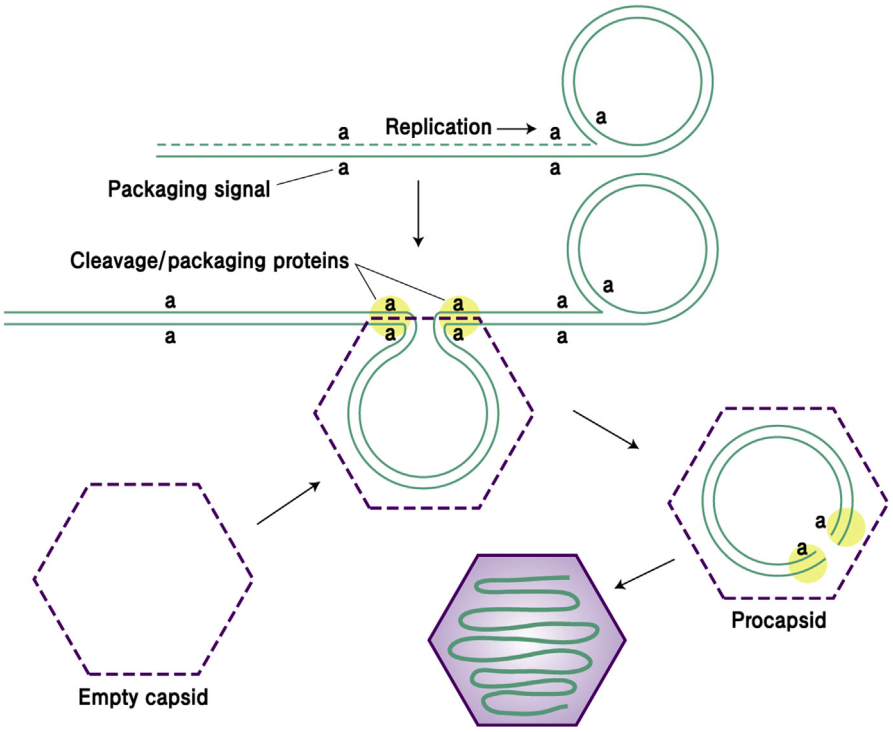


FIGURE 9.10 The steps involved in the viral DNA genome packaging. The viral genome replication is coupled with the genome packaging. The genome packaging is initiated by the recognition of "a" elements by the preassembled capsid. The cleavage of the concatemer by cleavage/packaging proteins is coupled with the DNA genome packaging. The sequence element "a" is denoted.

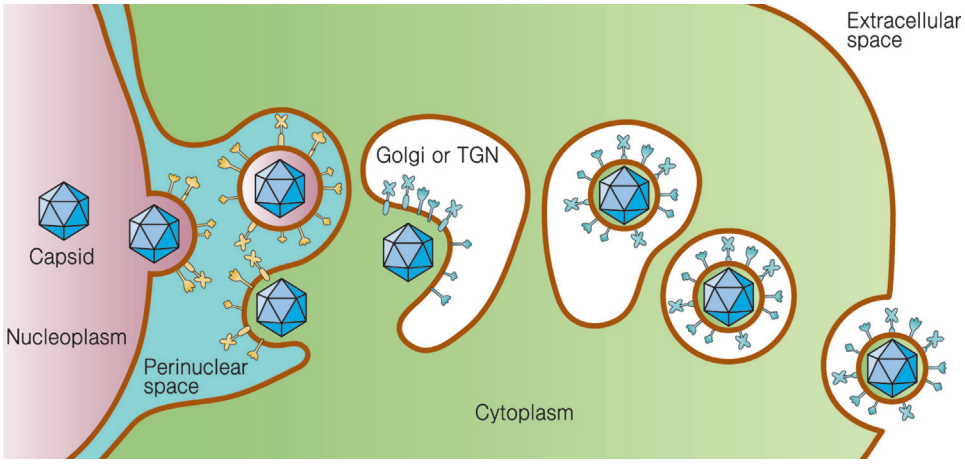


FIGURE 9.11 Nuclear egress and budding of HSV-1 particles. Nuclear egress of HSV-1 particle occurs through two sequential envelopment processes. The viral capsid assembled in the nucleus acquires its envelope via budding into perinuclear space (primary envelopment). Such enveloped particles lose its envelope via fusion with outer nuclear membrane. The capsid entered in cytoplasm acquires its envelope again via budding through Golgi or tans-Golgi network (TGN) (secondary envelopment) and then released.

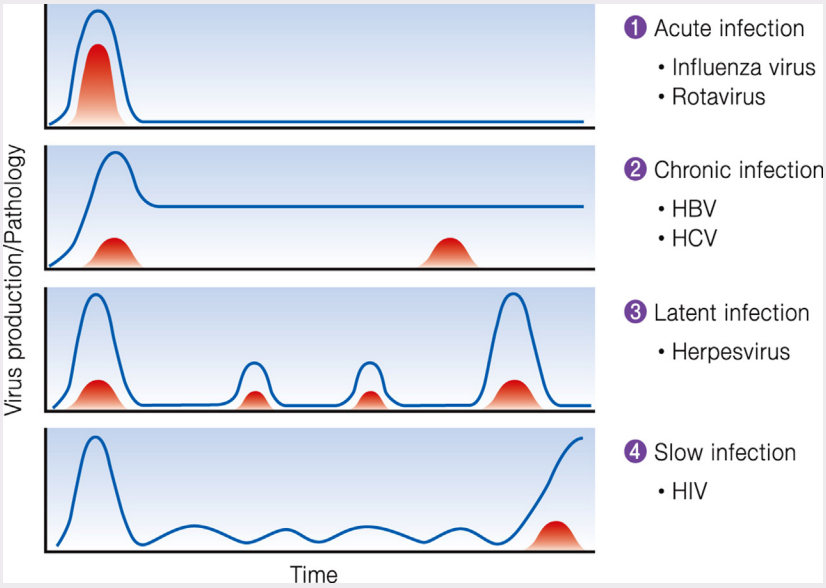
the Golgi or *trans*-Golgi network (TGN) (*secondary envelopment*). The enveloped virion is then transported in a vesicle to the plasma membrane for extracellular release. Importantly, some tegument proteins (as well as viral envelope glycoproteins) are obtained during secondary envelopment.

9.4 LATENT INFECTION

One salient feature of herpesvirus infection is "*latent infection*" (Box 9.1). In fact, primary infection of HSV-1 occurs in the *mucous layer* of the epithelium, while latent infection occurs mainly in the sensory neuron (Fig. 9.12). After productive primary infection, HSV-1 enters the sensory neuron axon and migrates along the axon to the cell body in a sensory ganglion of the periphery nervous system. In the sensory neuron cell body, the HSV-1 DNA is stably maintained as an episome in the nucleus. Thus, HSV-1 establishes a latent infection in the neuronal system deficient in immune cells. This residence in an immunologically privileged region is a way of *immune evasion* (see Table 5.4).

BOX 9.1 Patterns of Virus Infection

Outcomes of virus infection mainly rely on host immune response to virus infection. Depending on the extent of viral replication and pathogenesis during the course of infection, the pattern of viral infection can be divided into four kinds. First, depending on the viral persistence following primary infection, virus infection can be divided into two patterns: *transient infection* and *persistent infection*. In clinical circumstance, the former is often termed "*acute infection*," while the latter is termed "*chronic infection*." Acute infection refers as a viral infection limited by host immune response. Following primary infection, viruses are cleared from the body. Influenza virus and rotavirus infection are two representative examples. In contrast, chronic infection refers to a viral infection that evades host immune response and maintains viral replication for long periods. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are two representative examples. On the other hand, there are cases, where viral replication discontinuously occurs without clearance after primary infection. Depending on viral persistency, this discontinuous infection can be further divided into *latent infection* or *slow infection*. In the case of latent infection, viral replication occurs transiently during primary infection. Soon after, the virus stops replicating and becomes undetectable even in the circulating bloodstream, a period dubbed "*viral latency*." During latency, the viral genome is maintained without being eliminated and occasionally, the virus resumes its replication by a process termed "*reactivation*." Herpesvirus infection, such as HSV-1, is a representative of latent infection. The pattern of slow infection is similar to that of latent infection, but differs in that infection pathology becomes evident following long periods (ie, decades) of infection. Human immunodeficiency virus (HIV) infection is a representative of slow infection.



Four type of virus infection patterns. The extent of viral production (line) and the infection pathology (red) are drawn along with the progress of virus infection.

Once latent infection is established, the viral genome is maintained as a circular *episome*[9] without involving replication of the viral genome and production of progeny virus. During latency, HSV-1 limits gene expression to a single locus, the *latency-associated transcript (LAT)*. The role of LAT has been obscure, because of the lack of protein-coding. Four kinds of viral transcripts are detected that are all transcribed from the "**b**" element of the HSV-1 genome (Fig. 9.13). Evidence that LAT expresses any viral proteins is lacking. Intriguingly, LAT are processed to be *microRNA*.[10] Importantly, these miRNAs are shown to be critical for the maintenance of latency. In fact, two LAT-derived miRNAs suppress translation of ICP0 and ICP4 protein (see Fig. 9.13). Note that ICP0 and ICP4 are essential for viral early gene transcription (see Table 9.3). A long-standing puzzle about LAT has been resolved.

On the other hand, the virus in latency gets reactivated occasionally, and reenters the lytic phases of its life cycle, a process termed "*reactivation*." Although it is not clear yet as to what causes the reactivation, stimuli such as hormone, ultraviolet radiation, and stress are the suspects. Upon reactivation, the progeny virus trafficks via anterograde transport to peripheral tissues and induces lytic infection (see Fig. 9.12).

9. **Episome** A DNA that is stably present in the cell, excluding chromosomal DNA. The term "epi" is derived from Greek word for "above."
10. **MicroRNA** A kind of short RNA (ie, 20～22 nt in length) found in eukaryotic cells, that regulates mRNA translation and mRNA stability.

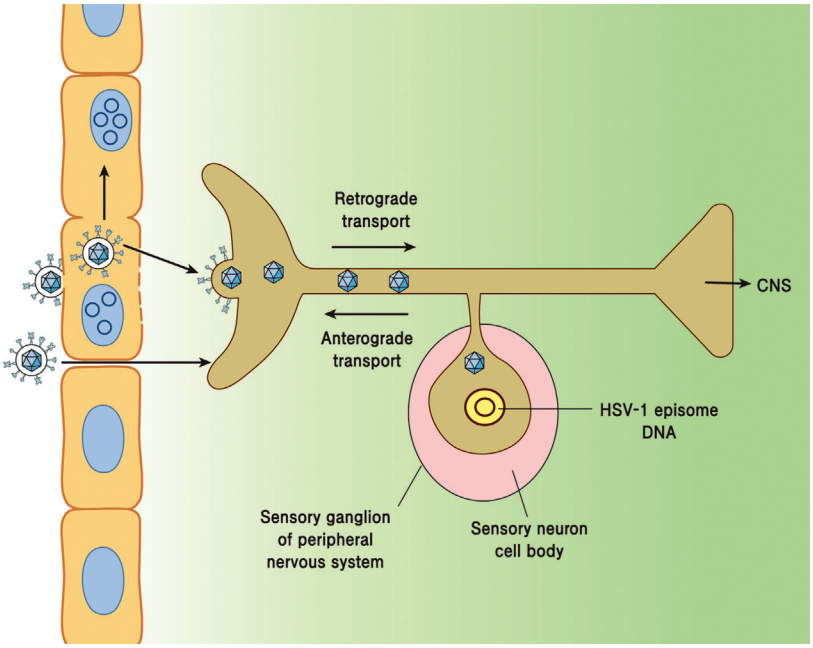


FIGURE 9.12 Viral spread to ganglion cells. Primary infection of HSV-1 occurs in the epithelial cell, where lytic infection is established. The viral particles released invade neuronal tissue by infecting nerve terminals in close contact. These axon terminals can derive from sensory neuron in dorsal root ganglia. The nucleocapsid is transported within axon to the neural cell body by microtubule-mediated transport, a process called "retrograde transport." Then, the viral DNA is delivered to the nucleus, where the viral episome is stably maintained, a hallmark of HSV-1 latency. Upon reactivation, the viral genome replication occurs and the resulting progeny capsid traffics via anterograde transport and gets to epithelial cells for lytic infection. *CNS*, central nervous system.

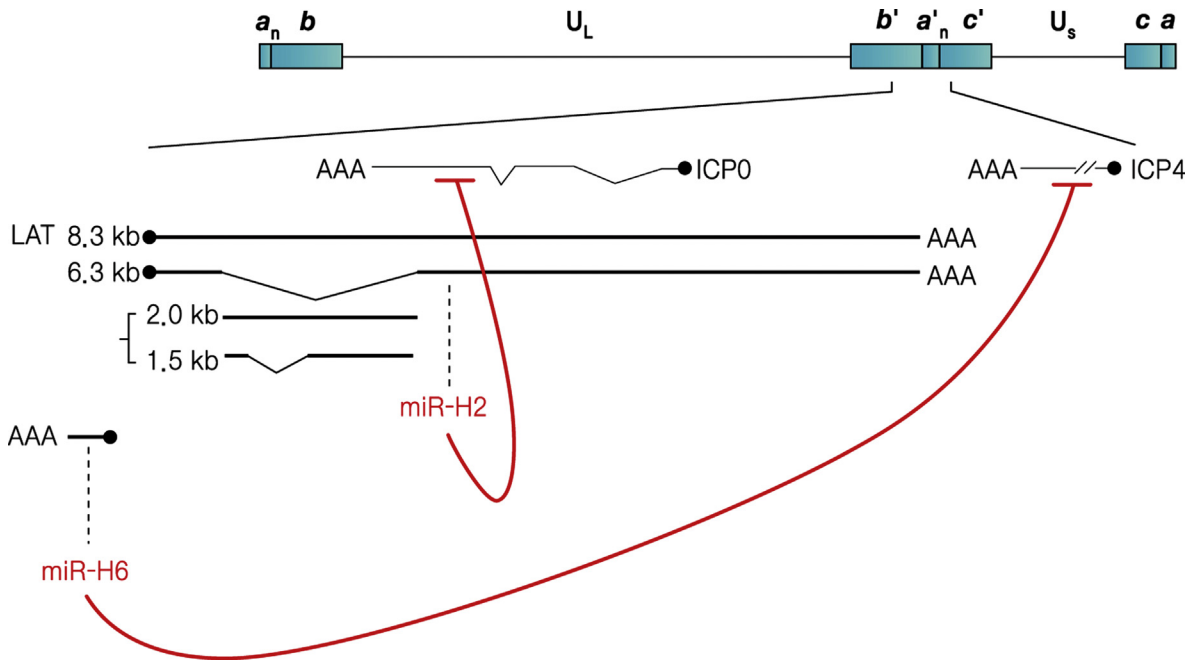


FIGURE 9.13 Latency-associated transcripts of HSV-1. The LAT gene is located in **b**′ and **a**′ elements within the IR region. Four LAT transcripts are found: two primary transcripts (ie, 8.3 and 2.0 kb) and two processed transcripts (ie, 6.3 and 1.5 kb), in which the introns are removed from corresponding primary transcripts. Several miRNAs are produced from the LAT region in the human trigeminal ganglia latently infected with HSV-1. In particular, miR-H2 miRNA, which is encoded within the second exon of the LAT, suppresses ICP0 gene expression, while miR-H6 miRNA, which lies in the opposite transcriptional orientation, just upstream of the LAT, suppresses ICP4 gene expression.

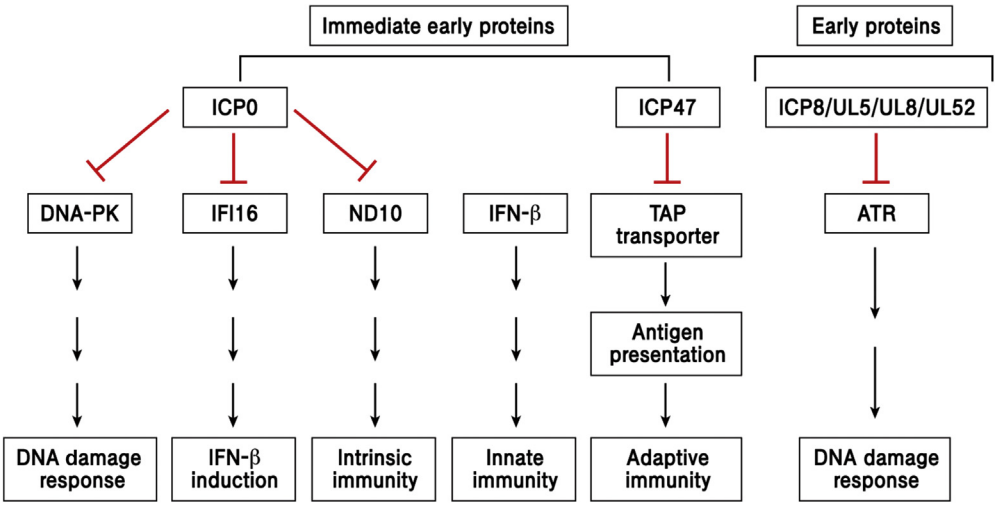


FIGURE 9.14 The effects of HSV-1 proteins on host functions. HSV-1 proteins (ie, ICP0, ICP47, and ICP8/UL5/UL8/UL52 complex) that subvert host functions are illustrated (see text). Arrowed lines indicate the activation, while the broken arrows indicate the inhibition. *DNA-PK*, DNA-dependent protein kinase.

9.5 EFFECTS ON HOST

HSV-1 infection significantly affects host cells in that it blocks DNA damage response signaling as well as host immunity.

Immunosuppression. HSV-1 can infect culture cells lytically. Nonetheless, only 37 genes out of 84 genes identified in the HSV-1 genome are essential for viral growth in cell culture, whereas the remaining 47 genes are dispensable. Since these seemingly dispensable genes are essential for the viral propagation in vivo, it is believed that these genes are required for immune regulation of the host immune response. In particular, the immunosuppressive roles of these genes, including ICP0, ICP47, Vhs, and ICP34.5, are beginning to be uncovered.

ICP0. Immediate-early protein, ICP0, is critical for counteracting the antiviral restriction. For instance, ICP0-null mutant is sensitive to interferon pretreatment in culture cells and is highly inhibited by interferon in vivo. Intriguingly, newly made ICP0 localizes in ND10 nuclear substructures, which are involved in "*intrinsic resistance*"[11] to HSV-1 infection (Fig. 9.14). Indeed, ICP0 targets several components of ND10, including PML protein, via an ubiquitin-mediated degradation; in doing so, it disrupts ND10 structures. ICP0 also targets *IFI16*[12] for degradation, which is implicated in IRF-3 activation. Overall, ICP0 counteracts host antiviral restriction (both intrinsic resistance and innate immunity) by degradation of ND10 components and IFI16 via its ubiquitin E3 ligase activity.

ICP47. Newly made viral antigens are presented on the cell surface as peptides by MHC class I molecules. This process, termed "antigen presentation," is critical for the adaptive immune response. ICP47 undermines adaptive immunity by inhibiting antigen presentation via binding to the *TAP transporter* (see Fig. 9.14). Specifically, ICP47 competes with antigenic peptides for the TAP binding site, thereby inhibiting TAP-dependent peptide translocation to ER (see Fig. 5.14).

ICP34.5. ICP34.5 is also known to suppress adaptive immunity. It turned out that ICP34.5 protein inhibits autophagy via its binding to beclin-1, which is essential for autophagy. Thus, autophagy-mediated MHC class II antigen presentation is blocked by ICP34.5.

DNA Damage Response. HSV-1 is a double-stranded DNA virus that replicates in the nucleus. The incoming viral DNA as a linear double-stranded DNA molecule is recognized as a double-strand DNA break (DSB), and as such must contend with cellular DNA damage response (DDR) (see Box 6.2). Both ATM- and ATR-mediated DDR are blocked in HSV-1 infected cells.

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11. **Intrinsic resistance** An antiviral response that is mediated by constitutively expressed cellular proteins, as opposed to induced proteins in innate immunity. It is also called intrinsic immunity.

12. **IFI16 (interferon gamma-inducible protein 16)** It is a type of pattern recognition receptors (PRRs) that recognizes the DNA genome of DNA viruses. It is also known as a "nuclear DNA sensor."

ICP0. First, ICP0 blocks *ATM-mediated DDR* by degradation of DNA-PK (protein kinase), which is activated in response to double-strand breaks (see Fig. 9.14).

ICP8. Secondly, the DNA damage response kinase, ATR, is specifically inactivated in HSV-1 infected cells. ATR and other associated factors, including ATRIP, RPA70, and Claspin, are recruited to the viral replication compartments in the nucleus. It appears that the viral DNA replication intermediates are recognized by ATR without concurrent activation. Recently, it was shown that ICP8 binds to ATR complex along with the helicase/primase complex (UL5/UL8/UL52), thereby inactivating ATR kinase. Overall, HSV-1 co-opts cellular DDR in that it utilizes some of ATR-mediated DDR machinery for efficient viral DNA replication without invoking the activation of ATR-mediated DDR.

9.6 CLINICAL SIGNIFICANCE OF HSV-1 INFECTION

Most adults are latently infected with multiple, at least two or three, herpesviruses (ie, HSV-1, VZV, and EBV), and are seropositive to these viral antigens. Herpesvirus infections do not cause serious diseases to healthy individuals. Nonetheless, the latently infected herpesviruses often cause serious sequels under certain circumstances such as in immunocompromised individuals. For instance, HSV-1 causes cold sores or watery blisters in the skin or mucous membranes in mouth and lips following reactivation. Treatment usually involves nucleoside analog antiviral drugs such as *acyclovir* that inhibits viral replication (see Fig. 9.1). Acyclovir is a *prodrug*,[13] which is converted into acyclovir triphosphate that acts as an inhibitor of the viral DNA polymerase (see Fig. 26.3). A preventive vaccine for HSV-1 is not available.

9.7 OTHER ALPHA-HERPESVIRUSES

Besides HSV-1, HSV-2 and VZV are also alpha-herpesviruses (see Table 9.2). HSV-2 is related to HSV-1, exhibiting over 50% genetic similarity. HSV-2 causes disorders in the reproductive organs, such as genital herpes. Genital herpes, known simply as *herpes*, is the second most common form of herpes. Acyclovir is used for the treatment, but a vaccine is not available.

On the other hand, VZV causes chicken pox to youngsters and herpes zoster to adults, which is often referred to as shingles (Fig. 9.15). Being transmitted via an airborne route, VZV is extremely contagious, and most people are infected before or during youth. Primary VZV infection to youngsters results in chicken pox (varicella), which may result in complications including encephalitis or pneumonia. After clinical symptoms of chicken pox are resolved, VZV still remains

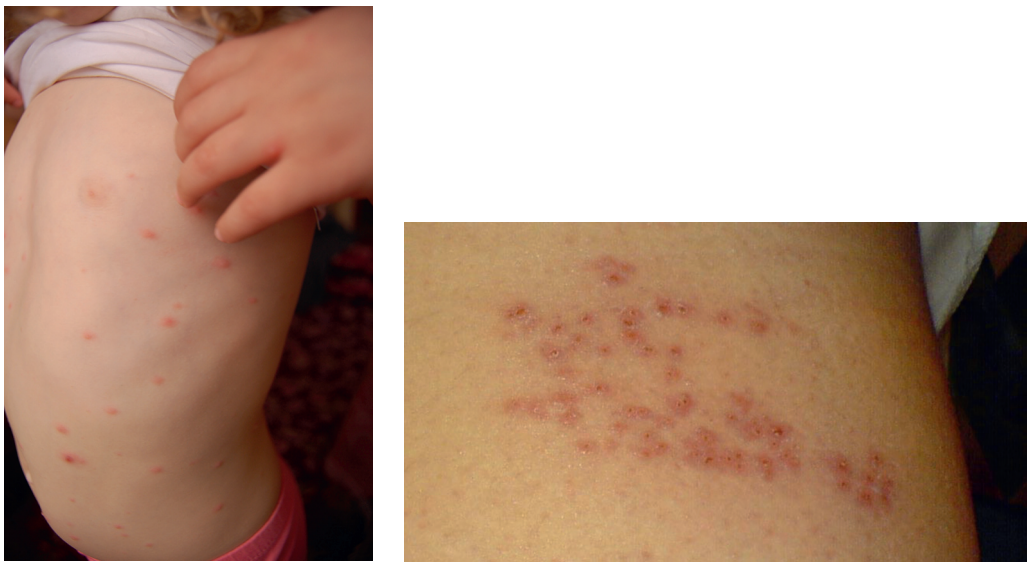

FIGURE 9.15 Photos of patients with varicellar and zoster. (Left) A 3-year-old girl with a chicken pox rash on her torso. (Right) Shingles blisters showing characteristic purple color.

13. **Prodrug** A prodrug is a medication that is administered in an inactive form, and then it becomes converted to its active form through a normal metabolic process.

BOX 9.2 Human Cytomegalovirus

HCMV (human cytomegalovirus) is a prototype of human beta-herpesviruses. Worldwide, over 80% of adults have been infected by HCMV, as indicated by the presence of antibodies. Although they may be found throughout the body, HCMV infections are frequently associated with the salivary glands. HCMV infection is typically unnoticed in healthy people, but can be life-threatening for the immunocompromised, such as HIV-infected persons and organ transplant recipients. In other words, HCMV is an *opportunistic pathogen*.[14] After infection, HCMV has an ability to remain latent within the body for long periods. HCMV is also the most frequently transmitted virus to a developing fetus. HCMV infection is more widespread in developing countries and is the most significant viral cause of birth defects in industrialized countries. Congenital HCMV is the leading infectious cause of deafness, learning disabilities, and mental retardation in children. Nucleoside analog drugs such as ganciclovir (see Fig. 26.1) are used for patients with life-threatening illnesses.

BOX 9.3 Epstein-Barr Virus

EBV (Epstein-Barr virus) is a prototype of human gamma-herpesviruses. Worldwide, over 90% of adults have already been infected by EBV. Infection with EBV occurs by oral transfer of saliva and genital secretions. EBV infects B cells of the immune system and epithelial cells. Once the virus's initial lytic infection is brought under control, EBV latently persists in the individual's B cells for the rest of the individual's life. EBV is best known as the cause of *infectious mononucleosis* (also known as "*mono*,") and sometimes being referred as the *kissing disease*. Most people are exposed to the virus as children, when the disease produces no noticeable or only flu-like symptoms. When infection with EBV occurs during adolescence, it causes infectious mononucleosis with frequency of 35–50%. The disease is manifested by fever, sore throat, and fatigue, along with several other possible signs and symptoms. It is generally a self-limiting disease, and little treatment is normally required. On the other hand, EBV is also associated with particular forms of cancer, such as *Burkitt's lymphoma*, and *nasopharyngeal carcinoma*. Importantly, Burkitt's lymphoma, the cancer of B lymphocytes, is geographically confined to malaria endemic regions in Africa, whereas nasopharyngeal carcinoma is also confined to Southern China. Hence, it is believed that EBV infection is a cofactor for carcinogenesis.

dormant in the nervous system of the infected person (virus latency), including the cranial nerve ganglia, dorsal root ganglia, and autonomic ganglia. VZV is often reactivated and causes a number of neurologic conditions, collectively termed "*Zoster*" or shingles. Unless properly treated at the earlier stage of infection, the consequence of zoster can be severe, resulting in complications such as "*postherpetic neuralgia*" often associated with dreadful pains and disability. Fortunately, effective medicines, such as acyclovir, are available for the treatment of herpes zoster. Two kinds of live attenuated VZV vaccines are available for the prevention of VZV infections: one for children to prevent chicken pox (ie, Varivax) and another more concentrated formulation for adults to prevent shingles (ie, Zostavax) (see Table 25.1)

Although alpha-herpesviruses are mainly described in this chapter for the sake of brevity, beta- and gamma-herpesviruses are equally important clinically. Here, HCMV, a prototype of beta-herpesviruses, and EBV, a prototype of gamma-herpesviruses, are briefly described with emphasis on clinical features (Boxes 9.2 and 9.3).

9.8 PERSPECTIVES

Herpesviruses stand out among human viruses in that they potently suppress the host immune response. Since herpesviruses have coexisted in human body for long time, it is believed that herpesviruses acquired multiple functions that are essential for maintaining latent infection during evolution. In this regard, herpesvirus has been an experimental model to investigate host immunity, in particular, antigen presentation. Some of the current issues relating herpesvirus research include the following. First, we need to better understand how the viral latency is maintained. Second, we need a better understanding of the biological significance of viral miRNAs. In addition to protein-coding genes, in fact, herpesviruses genome encodes quite a number of miRNAs, and intriguingly, some viral miRNAs derived from LAT control the reactivation of viral latency. Apparently, the viral miRNAs are critically important for the maintenance of

14. **Opportunistic pathogens** pathogens that usually do not cause disease in a healthy host, one with a healthy immune system, but do cause disease in compromised immune system.

latency. Any measures that control the LAT expression could be an effective strategy to block reactivation. Third, opportunistic pathogens, such as HSV-1, VZV, HCMV, and EBV, could become a serious threat to elderly persons as the aging population is increasing. Antiviral drugs that can prevent or control the reactivation of latently infected herpesviruses represent an unmet medical need. Finally, Kaposi's sarcoma-associated herpesvirus (KSHV) has been a focus of the last two decades, since KSHV is associated with Kaposi's sarcoma in AIDS patients. We have just begun to uncover some of the viral oncogenes involved in the viral carcinogenesis. Taken together, our better understanding of herpesvirus biology will help to prevent the sequels of the creeping viruses.

9.9 SUMMARY

- *Classification*: human herpesviruses are classified into three genera: alpha-, beta-, and gamma-herpesviruses. HSV-1 is a prototype of human alpha-herpesviruses.
- *Virion structure*: HSV-1 is an enveloped virus, in which the capsid is enclosed. Some tegument proteins, such as VP16, ICP0, and Vhs, are essential for the viral infection.
- *Genome*: HSV-1 genome represents a linear double-strand DNA, 150 kb in length, and it has a direct repeat element at both ends, termed terminal repeat or TR, that is essential for the viral genome replication.
- *Genome replication*: HSV-1 genome replication occurs largely via a rolling-circle mechanism and the DNA synthesis is driven by the virally encoded DNA polymerase.
- *Latency*: HSV-1 replicates primarily in epithelial cells but establishes latency in neuronal cells. The viral genome is maintained as a circular episome in latently infected neuronal cells. During latent infection, a latency-associated transcript or LAT is expressed.
- *Beta-herpesviruses*: HCMV, a prototype of human beta-herpesviruses, causes congenital disease and produces serious complications in immunocompromised individuals.
- *Gamma-herpesviruses*: EBV, a prototype of human gamma-herpesviruses, is a human tumor virus in that it is associated with Burkitt's lymphoma, and nasopharyngeal carcinoma.

STUDY QUESTIONS

9.1 An immediate early protein, ICP0, is important for the regulation of lytic and latent viral infection. ICP0-null mutants could not lead to lytic infection in human diploid fibroblasts (primary cells), while it could lead to lytic infection in certain cell lines such as HeLa cells and U2OS cells. Explain why?

9.2 Compare the similarity and difference between two modes of HSV-1 DNA replication.

9.3 LAT was shown to be processed to miRNAs that regulate reactivation. What are the advantages of using miRNA, as opposed to mRNA (protein-coding genes) in a viral perspective?

SUGGESTED READING

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JOURNAL CLUB

- Mohni, K.N., Smith, S., Dee, A.R., Schumacher, A.J., Weller, S.K., 2013. Herpes simplex virus type 1 single strand DNA binding protein and helicase/primase complex disable cellular ATR signaling. *PLoS Pathog.* 9(10), e1003652.

  Highlight: HSV-1 has evolved to disable the cellular DNA damage response kinase, ATR. This article demonstrated that the HSV-1 single strand binding protein (ICP8) and the helicase/primase complex (UL8/UL5/UL52) form a nuclear complex in transfected cells that is necessary and sufficient to disable ATR signaling. The data suggested that these four viral proteins prevent ATR activation by binding to the DNA substrate and obscuring the loading of cellular factors. This is the first example of viral DNA replication proteins obscuring access to DNA substrate that would normally trigger DNA damage response.