CHAPTER 18

Replication of Some Nuclear-replicating Eukaryotic DNA Viruses with Large Genomes

The term *large*, when applied to DNA virus genomes, must be relative. The genomes of large DNA viruses encode anywhere from 50 to more than 250 distinct genes, and on the upper end of size, the viral genomes approach the size of the simplest "free-living" organisms: the mycoplasmas.

Much of the genetic complexity of large, nuclear-replicating DNA viruses is due to viral genes devoted to providing the virus with the ability to replicate and to mature in differentiated cells, as well as viral defenses against or accommodations to host defense mechanisms. These genes are often not required for virus replication in one or another type of cultured cells, at least under certain conditions, and have been termed "dispensable for virus replication." This designation is in relatively common use but is misleading, because no virus gene maintained in a wild strain that replicates efficiently in the population at large is dispensable.

Stripped of "dispensable" genetic functions, a large-genome DNA virus must contain the same essential components as one with a small genome: genes devoted to subverting the cell into a virus-specific transcription factory, enzymes for viral genome replication, and the proteins and enzymes required to form the capsid and to assemble and release new infectious virions. Given these requirements, it is not too surprising that the replication basics of these large-genome, nuclear-replicating DNA viruses follow the same basic strategies as seen with smaller DNA viruses.

It is important to keep in mind, however, that there are many different ways a virus can modify a cell to result in a site favorable for its replication—"the devil is in the details"!

HERPESVIRUS REPLICATION AND LATENCY

The herpesviruses as a group

General features

The herpesviruses are extremely successful enveloped DNA viruses. They have been identified in all vertebrate species studied, and extend into other classes of the animal kingdom (oysters, for example). Their replication strategy involves a close adaptation to the immune defense of the host, and it is possible that their evolutionary origins as herpesviruses lie in the origins of immune memory. Eight discrete human herpesviruses are described; each causes a characteristic disease.

Many herpesviruses are neurotropic (i.e., they actively infect nervous tissue); all such viruses are collectively termed *alpha-herpesviruses*. Three human herpesviruses belong to this group: the closely related herpes simplex virus types 1 and 2 (HSV-1 and -2), which are the primary agents of recurrent facial and genital herpetic lesions, respectively; and varicella-zoster virus (VZV), which is the causative agent of chicken pox and shingles. VZV is more distantly related to HSV.

Five human herpesviruses are lymphotropic, meaning that they replicate in tissues associated with the lymphatic system. These herpesviruses have been subdivided into beta- and gamma-herpesvirus groups based on the specifics of their genome structure and replication. Viruses in these two groups share features that suggest they are more closely related to each other than they are to the three neurotropic herpesviruses.

Infections with human cytomegalovirus (HCMV) (the prototype of beta-herpesviruses) are linked both to a form of infectious mononucleosis and to congenital infections of the nervous system. This virus can be devastating in individuals with impaired immune function, such as those suffering from AIDS or being clinically immune suppressed for organ transplantation.

Infections with two other lymphotropic herpesviruses—the closely related beta-human herpesviruses-6 and -7 (HHV-6 and HHV-7)—are generally mild early-childhood diseases.

Infections with human gamma-herpesviruses, Epstein-Barr virus (EBV) and the recently described Kaposi's sarcoma herpesvirus or human herpesvirus-8 (KSHV or HHV-8), are convincingly linked to human cancers. Despite the high frequency of EBV infection in the general population, carcinogenesis is linked to additional environmental factors, and the infection in most humans is either asymptomatic or results in a form of mononucleosis that is very similar in course to that caused by HCMV.

Genetic complexity of herpesviruses

Typically, a herpesvirus genome contains between 60 and 120 genes. Unlike adenoviruses, all of which share a basic genomic structure as well as general architecture, a comparative survey of the various herpesviruses' genomic structures displays a staggering array of individual variations on a general theme. Still, within this variation, gene order is generally maintained within large blocks of the genome and varying degrees of genetic homology are clearly evident. The most striking areas of homology are seen among those genes that provide basic replication functions.

One general feature of the complex herpesvirus genome arrangement is that herpes genomes contain significant regions of inverted repeat sequences. The size of herpesvirus genomes varies from 80 kbp to 240 kbp. Given that all the viruses share basic features of productive infection, this range in size means that different herpesviruses differ greatly in the number of "dispensable" genes they encode that are devoted to specific aspects of the pathogenesis and spread of the virus in question. Examples of such differences are described a bit further along in this chapter.

Common features of herpesvirus replication in the host

The replication strategies of all herpesviruses appear to share some basic features. The viruses establish a primary infection during which virus replicates to moderate or high titers, yet with generally mild symptoms that are fairly rapidly resolved. One outcome of this primary infection in the host is efficient and effective immunity against reinfection.

The virus is not completely cleared from the host, however. Instead, one or another specific cells infected by the virus are able to maintain the viral genomes without a productive virus infection. This maintenance is a result of the virus' being dependent upon specific cellular transcriptional machinery for high efficiency replication. The presence of critical components of this machinery is highly dependant upon the state of differentiation and the intercellular environment of cells in those tissues in which the virus replicates and establishes latency. As with other DNA viruses that exhibit a similar pattern of persistence without apparent active infection, this is termed a *latent infection*.

While definitions of *latency* vary with the virus in question, the strictest definition (which can be readily applied to herpesvirus latency) requires that no infectious virus be detectable in the host during the latent phase.

With appropriate stress to those cells harboring virus along with stress to the host's immune system, the activity of critical components of the cell's transcriptional machinery is activated, and virus can reactivate from latently infected tissue. Provided host immunity is sufficiently suppressed, a generally milder version of the primary infection ensues. This reactivation results in virus being available for infection of immunologically naive hosts, and establishes the infected individual as a reservoir of infection for life.

Since the major groups of herpesviruses have evolved to utilize different terminally differentiated cell types as a reservoir in which virus replication must occur at some low level to initiate recrudescence, it follows that those viral genes devoted to the ability of the virus to replicate in the immune competent host will show much divergence. At the same time, the basic similarity of the productive replication cycle, once it occurs, suggests that—as is the case—those viral genes involved in high titer replication will be recognizably similar.

The replication of HSV

The HSV virion

All herpesviruses possess similar enveloped icosahedrons. The envelope of HSV contains 10 or more glycoproteins. The matrix (called the *tegument* for obscure reasons) lies between the envelope and the capsid and contains at least 15 to 20 proteins. The capsid itself is made up of six proteins; the major one, VP5, is the 150 kd major capsid protein. VP5 is also called U_L19 for the position of its gene on the viral genetic map. A computer-enhanced model of the HSV capsid structure is shown in Fig. 9.3. A more conventional electron microscopic view is shown in Fig. 18.1. The molar ratio of HSV capsid proteins is tabulated in Table 11.2—various capsid proteins are present in widely differing amounts.

The viral genome

While each herpesvirus is different, a number of general features can be illustrated with the HSV-1 genome. The HSV-1 genome is linear, and is 152,000 base pairs (bp) long. With HSV, the left end of the genome is set as 0 map unit and the right is 1.00 map unit; therefore, each 0.1 map unit is 15,200 bp. Although the virion DNA is linear, the genome becomes circular upon infection. An electron micrograph of this DNA, which is about 50 microns long, is shown in Fig. 11.9.

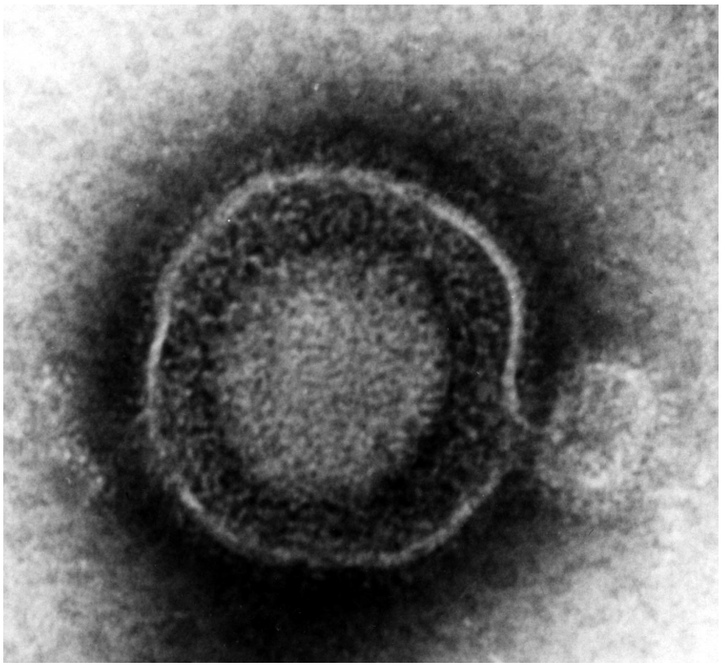

Fig. 18.1 Electron micrograph of an enveloped HSV-1 virion revealing specific features, especially glycoprotein spikes projecting from the envelope. The capsid has a diameter of about 100 nm and encapsidates the 152,000 bp viral genome. The interior of the capsid does not contain any cellular histones, in contrast to smaller DNA viruses. Rather, it contains relatively high levels of polyamines such as spermidine and putrescine, which serve as counterions to allow compact folding of the viral DNA needed in the packaging. (Photograph courtesy of Jay Brown.)

A high-resolution genetic and transcription map of the HSV genome is shown in Fig. 18.2. Because the genome becomes circular, the map is shown as a circle, but note that the genome's ends are indicated at the top of the circle. Since the virus encodes nearly 100 transcripts and more than 70 open translational reading frames (ORFs), the map is complex. Still, the basic methods of interpreting it are the same as with the simpler SV40 map. Interpretation of the HSV genetic and transcription map is aided by the fact that few viral transcripts are spliced and most ORFs are expressed by a single transcript, each with a contiguous promoter.

The genetic map of HSV-1 is summarized in Table 18.1, where viral proteins and other genetic elements are listed. The number of viral proteins that are not required for replication of the virus in cultured cells is large. Many of these dispensable proteins have a role in aspects of the pathogenesis of the virus. The exact function of such proteins, in theory, can be established by studying the effect of the deletion of the genes encoding them on the way the virus replicates in its natural host. Because the natural host of HSV is humans, this analysis must be carried out in animal models. This study can be a difficult task, and the actual biological functions of many virus-encoded proteins and enzymes are still unknown. The genome can be divided into six regions, each encoding a specific function as follows:

1 The ends of the linear molecules. The ends of the genome contain repetitive DNA sequences made up of various numbers of repeats of three basic patterns or groupings termed "a," "b," and "c." The "a" sequences also are found at the junction between the long and short segments of the genome (see a later section). They also contain the signals used in the assembly of mature virions for packaging of the viral DNA.

2 The long repeat (R_L) region. The 9000 bp repeat (R_L) encodes both an important immediate-early regulatory protein ($\alpha 0$) and the promoter of most of the "gene" for the latency-associated transcript (LAT). This transcript functions in reactivation from latency by an as yet unknown mechanism.

3 The long unique (U_L) region. The long unique region (U_L), which is 108,000 bp long, encodes at least 56 distinct proteins (actually more because some ORFs are spliced and expressed in redun-

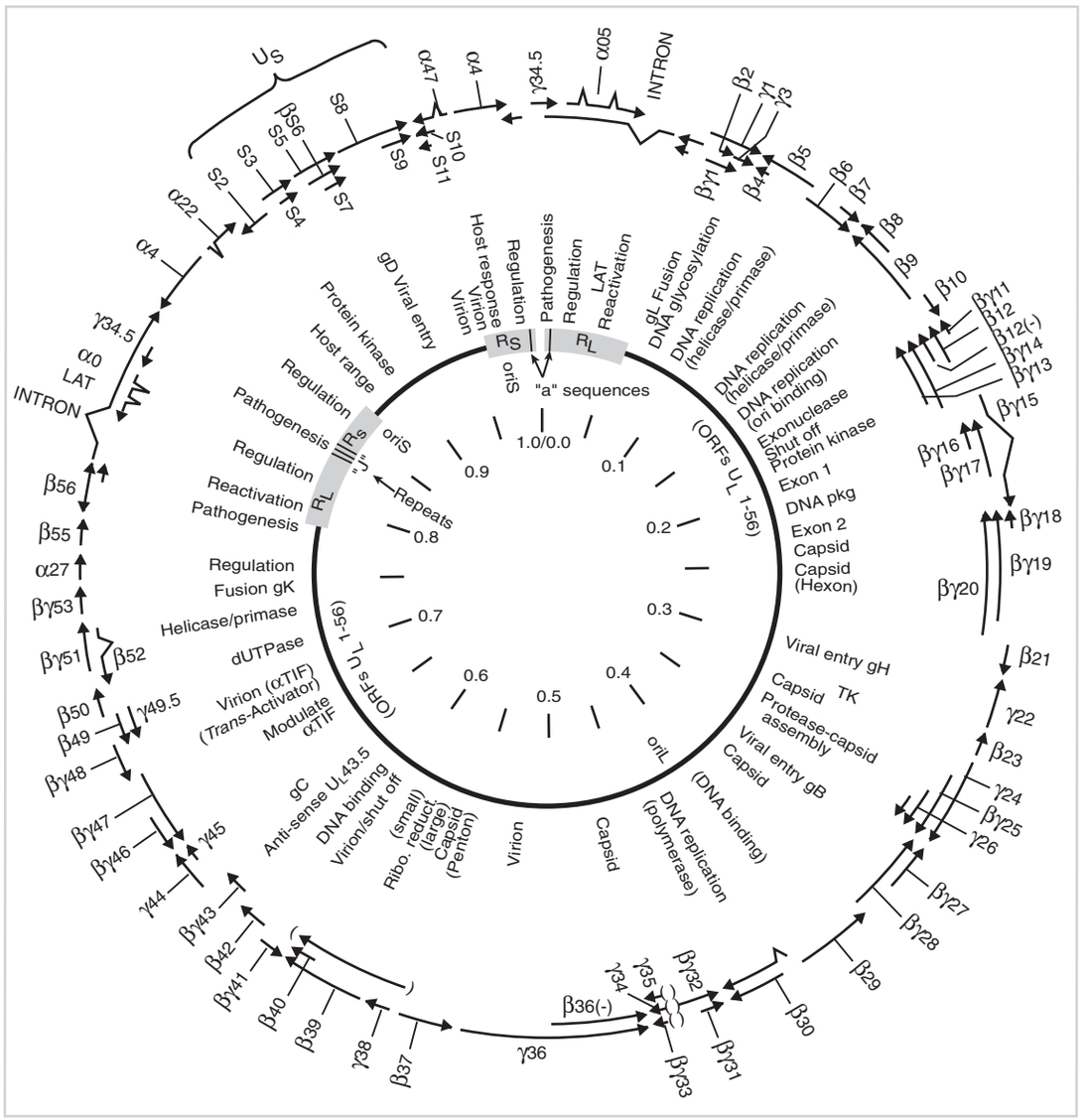

Fig. 18.2 The HSV-1 genetic and transcription map. Specific features of the genome are discussed in the text, and tabulated in Table 18.1. Individual transcripts are controlled by their own specific promoters, and splicing is uncommon. Each transcript is headed by its own promoter, and most are terminated with individual cleavage/polyadenylation signals. The time of expression of the various transcripts is roughly divided into immediate-early (α), early (β), late (βγ), and strict late (γ). This is, in turn, based on whether the transcripts are expressed in the absence of viral protein synthesis (α), before viral DNA replication and shutoff following this (β), before viral DNA replication but reaching maximum levels following this (βγ), or only following viral DNA replication (γ). The genome is about 152,000 bp and contains extensive regions of duplicated sequences.

dant ways). It contains genes for the DNA replication enzymes and the capsid proteins, as well as many other proteins.

4 The short repeat (R_S) regions. The 6600 bp short repeats (R_S) encode the very important α4 immediate-early protein. This is a very powerful transcriptional activator. It acts along with α0 and α27 (in the U_L region) to stimulate the infected cell for all viral gene expression that leads to viral DNA replication.

Table 18.1 Some genetic functions encoded by Herpes Simplex Virus Type 1.

Location (map unit) (Fig. 18.2)	Required for replication in culture?	Name of element or protein	Function
0.0	Yes	"a"	*Cis* genome cleavage, packaging signal
0.00–0.06	Yes	R_L	See below
0.05	No	ICP34.5	Reactivation (?)
0.01 (R_L)	Yes	α0	Immediate-early transcription regulator (mRNA spliced)/interferon inhibitor
0.02 (R_L)	No	LAT	Approximately 600 bases in 5′ region facilitate reactivation; no protein involved
0.04 (R_L)	No	LAT-intron	Stable accumulation in nucleus of latently infected neurons, unknown function
0.06	Yes	gL	Viral entry, associates with gH
0.07	No	U_L2	Uracil DNA glycosylase, DNA repair
0.08	No	U_L3	Nonvirion membrane-associated protein
0.09	No	U_L4	Tegument protein, unknown function
0.1	Yes	Helicase-primase	DNA replication
0.1	Yes	U_L6	Capsid protein, capsid maturation, DNA packaging
0.11	No	U_L7	Unknown
0.12	Yes	Helicase-primase	DNA replication
0.13	Yes	Ori-binding protein	DNA replication
0.14	No	gM	Glycoprotein of unknown function
0.14	Yes	U_L11	Tegument protein, capsid egress and envelopment
0.16	Yes	Alkaline exonuclease	DNA packaging (?), capsid egress
0.15	No	$U_L12.5$	C-terminal two-thirds of U_L12, expressed by separate mRNA; specific function unknown
0.17	No	Protein kinase	Tegument associated
0.18	No	U_L14	Unknown
0.16/0.18	Yes	U_L15	DNA packaging, cleavage of replicating DNA (?), (spliced mRNA)
0.17	No	U_L16	Unknown
0.2	Yes	U_L17	Cleavage and packaging of DNA
0.23	Yes	Capsid	Triplex
0.25	Yes	Capsid	Major capsid protein, hexon
0.27	Yes	U_L20	Membrane associated, virion egress
0.28	No	U_L21	Tegument
0.3	Yes	gH	Viral entry, functions with gL

Table 18.1 *Continued*

Location (map unit) (Fig. 18.2)	Required for replication in culture?	Name of element or protein	Function
0.32	No	U_L23	Thymidine kinase
0.33	No	U_L24	Unknown
0.33	Yes	U_L25	Tegument protein, capsid maturation, DNA packaging
0.34	Yes	U_L26	Maturational protease
0.34	Yes	$U_L26.5$	Scaffolding protein
0.36	Yes	gB	Glycoprotein required for virus entry
0.37	Yes	U_L28	Capsid maturation, DNA packaging
0.4	Yes	U_L29	ssDNA-binding protein, DNA replication
0.41	No	Ori_L	Origin of replication
0.42	Yes	DNA pol	DNA replication
0.45	No	U_L31	Nuclear phosphoprotein, nuclear budding
0.45	Yes	U_L32	Capsid maturation, DNA packaging
0.46	Yes	U_L33	Capsid maturation, DNA packaging
0.47	No	U_L34	Membrane phosphoprotein, nuclear budding
0.47	Yes	U_L35	Capsid protein, capsomer tips
0.50	No	U_L36	ICP1/2, tegument protein
0.55	No	U_L37	Tegument phosphoprotein
0.57	Yes	U_L38	Capsid protein, triplex
0.58	Yes	U_L39	Large-subunit ribonucleotide reductase
0.59	Yes	U_L40	Small-subunit ribonucleotide reductase
0.6	No	U_L41	VHS, virion-associated host shutoff protein, destabilizes mRNA, envelopment
0.61	Yes	U_L42	Polymerase accessory protein, DNA replication
0.62	No	U_L43	Unknown
0.62	No	$U_L43.5$	Antisense to U_L43
0.63	No	gC	Initial stages of virus–cell association
0.64	No	U_L45	Membrane associated
0.65	No	U_L46	Tegument associated, modulates α-TIF
0.66	No	U_L47	Tegument associated, modulates α-TIF
0.67	Yes	α-TIF	Virion-associated transcriptional activator, enhances immediate-early, envelopment transcription through cellular Oct-1 and CTF binding at TATGARAT sites

Table 18.1 *Continued*

Location (map unit) (Fig. 18.2)	Required for replication in culture?	Name of element or protein	Function
0.68	No	U_L49	Tegument protein
0.68	No	U_L49.5	Unknown
0.69	No	dUTPase	Nucleotide pool metabolism
0.7	No	U_L51	Unknown
0.71	Yes	Helicase/primase	DNA replication
0.73	No	gK	Virion egress
0.74	Yes	α27	Immediate-early regulatory protein, inhibits splicing
0.75	No	U_L55	Unknown
0.76	No	U_L56	Tegument protein, affects pathogenesis
0.76 to 0.82	Yes	R_L	See R_L above
0.82	Yes	R_L/R_S Junction	Joint region, contains "a" sequences
0.82–0.86	Yes	R_S	See below
0.82–0.86 (R_S)	Yes	α4	Immediate-early transcriptional activator
0.86 (R_S)	Yes	Ori_S (*cis*-acting)	Origin of replication
0.86	No	α22	Immediate-early protein, affects virus's ability to replicate in certain cells
0.87	No	U_S2	Unknown
0.89	No	U_S3	Tegument-associated protein kinase, phosphorylates U_L34 and U_S9
0.9	No	gG 4	Glycoprotein of unknown function
0.9	No	gJ	Glycoprotein of unknown function
0.91	Yes	gD	Virus entry, binds HVEM
0.92	No	gI	Glycoprotein that acts with gE, binds IgG-Fc, and influences cell-to-cell spread of virus
0.93	No	gE	Glycoprotein that acts gI, binds IgG-Fc, and influences cell-to-cell spread of infection
0.94	No	U_S9	Tegument-associated phosphoprotein
0.95	No	U_S10	Tegument-associated protein
0.95	No	U_S11	Tegument-associated protein phosphoprotein, RNA binding, post-transcriptional regulation
0.96	No	α47	Immediate-early protein that inhibits MHC class I antigen presentation in human and primate cells
0.96–1.00	Yes	R_S	See R_S above
1	Yes	"a"	*Cis* genome cleavage, packaging signal

5 The origins of replication (ori's). HSV contains three short regions of DNA that serve as ori's. In the laboratory, any two can be deleted and virus replication will occur, but the three ori's are always found in clinical isolates. Ori_L is in the middle of the U_L region; ori_S is in the R_S, and thus, is present in two copies. All sets of ori's operate during infection to give a very complicated network of concatemeric DNA and free ends in the replication complex.
6 The unique short (U_S) region. The 13,000 bp unique short region (U_S) encodes 12 ORFs, a number of which are glycoproteins important in viral host range and response to host defense. This region also encodes two other proteins, α22 and α47, which are expressed immediately upon infection. The latter serves to block the infected cell's ability to present viral antigens at its surface.

HSV productive infection

HSV has a very complex genome, and the herpesviruses are the first ones described that have diploid copies of some of their genes. Still, the pattern of productive infection is roughly similar to that seen for smaller DNA viruses. In an HSV infection, the virus supplies most of the components it needs to replicate; each HSV gene is encoded by an mRNA that has its own promoter and polyadenylation signal. Most (but not all) HSV transcripts are unspliced and the relationship between gene structure and encoded polypeptide is relatively simple. Each protein tends to encode only one function, in contrast to the infection cycle of smaller viruses where a single protein may have many functions.

During the productive replication (**vegetative**) cycle, HSV gene expression is characterized by a progressive *cascade* of increasing complexity where the earliest genes expressed are important in "priming" the cell for further viral gene expression, in mobilizing cellular transcriptional machinery, and in blocking immune defenses at the cellular level. This phase is followed by the expression of a number of genes that are either directly or indirectly involved in viral genome replication. And, finally, upon genome replication, viral structural proteins are expressed in high abundance.

The time required for completion of a replicative cycle of HSV and other alpha-herpesviruses is fast compared with beta- and gamma-herpesviruses as well as smaller nuclear-replicating DNA viruses such as adenoviruses and papovaviruses. HSV is able to replicate in a wide selection of animals, tissues, and cultured cells.

Initial steps in infection: virus entry The process of HSV infection and transport of viral DNA to the nucleus is shown in outline in Fig. 18.3a. Virus attachment and entry require sequential interactions between specific viral membrane glycoproteins and cellular receptors. A group of related receptors have been recently identified and are termed *herpesvirus entry mediators*, or HVEMs. Based on sequence analysis, these proteins, which occur widely but in varying proportions in different cell types, are related to cellular proteins that interact with the tumor necrosis factor and the poliovirus receptor. Their function in the uninfected cell is unknown.

The virion membrane fuses with the host cell's membrane and capsid, and some tegument proteins are transported to the nucleus along cellular microtubules. The initial stages of infection and the fate of the viral envelope are shown in Fig. 6.3. The virion-associated host shutoff protein (vhs, or U_L41) appears to remain in the cytoplasm where it causes the disaggregation of polyribosomes and degradation of cellular and viral RNA.

Unlike the genomes of smaller nuclear-replicating eukaryotic viruses, the HSV genome is not encapsidated with and does not associate with cellular chromosomal proteins. Upon entry into the cytoplasm, the nucleocapsid is transported to the nuclear pores, where viral DNA is released into the nucleus. Elegant electron micrographs showing the docking and release of viral genome into the nucleus are seen in Fig. 18.3b. The viral genome is accompanied by the **α-TIF** protein (alpha-*trans*-inducing factor protein; also called VP16 or U_L48), which functions in enhancing immediate-early viral transcription. It does this by interacting with cellular proteins such

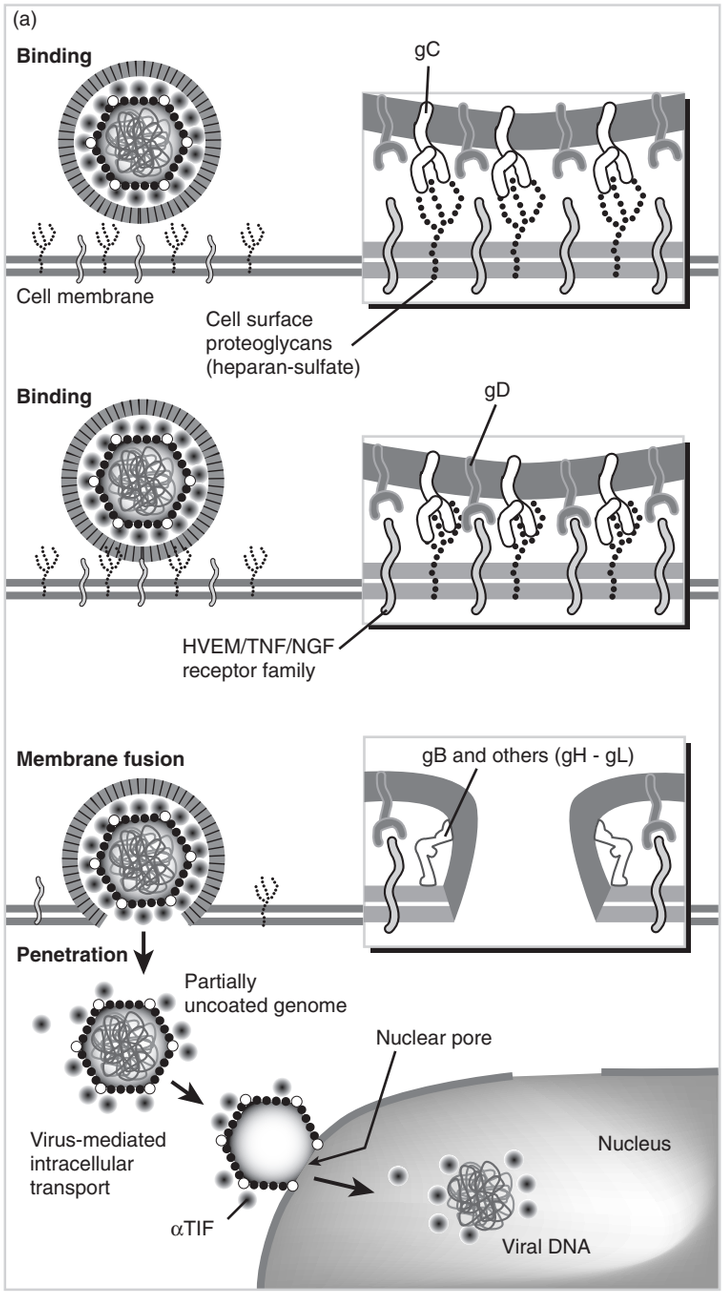

Fig. 18.3 The entry of HSV-1 into a cell for the initiation of infection. *a.* Outline of the process. The initial association is between proteoglycans of the surface and glycoprotein C (gC); this is followed by a specific interaction with one of several cellular receptors collectively termed herpesvirus entry mediators (HVEMs). These are related to receptors for nerve growth factors (NGFs) and tumor necrosis factor (TNF). The association requires the specific interaction with glycoprotein D (gD). Fusion with the cellular membrane follows; this requires the action of a number of viral glycoproteins including gB, gH, gI, and gL. An electron micrographic study of herpesvirus fusion with the infected cell is shown in Fig. 6.3. The viral capsid with some tegument proteins then migrate to nuclear pores utilizing cellular transport machinery. This "docking" is thought to result in the viral DNA being injected through the pore while the capsid remains in the cytoplasm. Some tegument proteins, such as α-TIF, also enter the nucleus with the viral genome. *b.* Electron micrographic analysis of pseudorabies virus capsid "docking" and genome injection at the nuclear pore. A logical sequence is shown progressing from the (dark) full capsid to an empty one. The process is quite similar to injection of bacteriophage DNA into a bacterial cell (see Chapter 6). (Micrographs reprinted with the kind permission of the American Society for Microbiology from Granzow, H., Weiland, F., Jons, A., Klupp, B. G., Karger, A., and Mettenleiter, T. C. Ultrastructural analysis of the replication cycle of pseudorabies virus in cell culture: A reassessment. *Journal of Virology* 1997;71:2072–82.)

as **oct1** (octamer-binding protein). Interestingly, it is the cellular protein that binds to the specific HSV-1 immediate-early gene promoter enhancer! The binding is to an 8 base stretch of nucleotides that has the nominal sequence TATGARAT where R represents any purine.

Immediate-early gene expression The process of viral gene expression during productive infection is schematically shown in Fig. 18.4. It can be subdivided into a number of stages starting with immediate-early gene expression. This stage of infection is also termed the "alpha" phase of gene expression, and is functionally similar to the immediate or pre-early stage of gene E1A and E1B

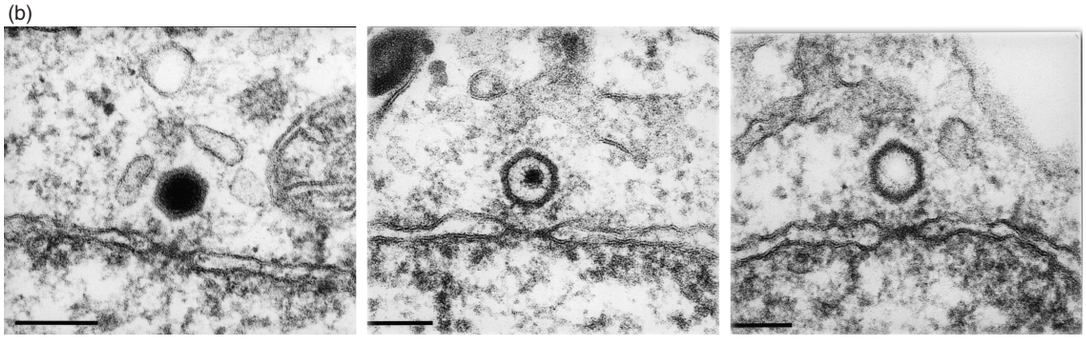

Fig. 18.3 *Continued*

expression in adenovirus infection. Five HSV genes (α4–ICP4, α0–ICP0, α27–ICP27/U_L54, α22–ICP22/U_S1, α47–ICP47/U_S12) are expressed and function in this earliest stage of the productive infection cycle.

In HSV infection, immediate-early transcription is mediated by action of the virion tegument (matrix) protein α-TIF through its interaction with cellular DNA-binding proteins at specific enhancer elements associated with individual alpha-transcript promoters. The α-TIF protein is an extremely powerful transcriptional activator with very broad specificity. Its C-terminal region contains a very large number of acidic amino acids that activate transcription by mobilizing RNA polymerase bound to the pre-initiation complex at the promoter in the vicinity of the enhancer region. The activator is tethered to the DNA in this region by interaction between cellular DNA-binding proteins and elements within its N-terminal domain. This type of transcriptional activator is termed an **acid blob activator**, and α-TIF is the prototype of the group.

The result of this interaction between viral transcription factors and cellular DNA-binding proteins is that even in a cell that is not transcriptionally active, such as one that is not actively replicating, the virus can stimulate expression of its own genes. This is an alternative and highly regulated counterexample to the induction of cellular DNA synthesis and associated metabolic activation carried out by papovaviruses and adenoviruses through interaction of their early (or immediate-early) gene products with cellular growth regulators.

Three of the proteins encoded by the immediate-early HSV genes—the α4, α0, and α27 proteins—are transcriptional regulators and activators of broad specificity. They function throughout the replication cycle. The mechanism of action of these transcriptional activators is complex.

The α4 protein appears to interact with the basal transcription complexes forming at the TATA boxes of viral (and cellular) promoters and making the process of initiation of transcription more efficient.

The α0 protein does not bind directly to DNA and part of its function may be to mobilize cellular transcriptional machinery by induction of structural changes to the organization of the host cell nucleus.

The α27 protein exhibits a number of functions, including mediating the transport of unspliced viral mRNA from the nucleus to the cytoplasm, inhibiting cellular splicing, influencing polyadenylation site usage, and activating transcription by an unknown mechanism.

The two other α proteins, α22 and α47, are dispensable for virus replication in many types of cultured cells. But α22, which has a role in the posttranscriptional processing of some transcripts, is required for HSV replication in some cell types and may have a role in maintaining the virus's ability to replicate in a broad range of cells in the host. Perhaps this is achieved by providing some types of cells with the capacity to express a group of late transcripts.

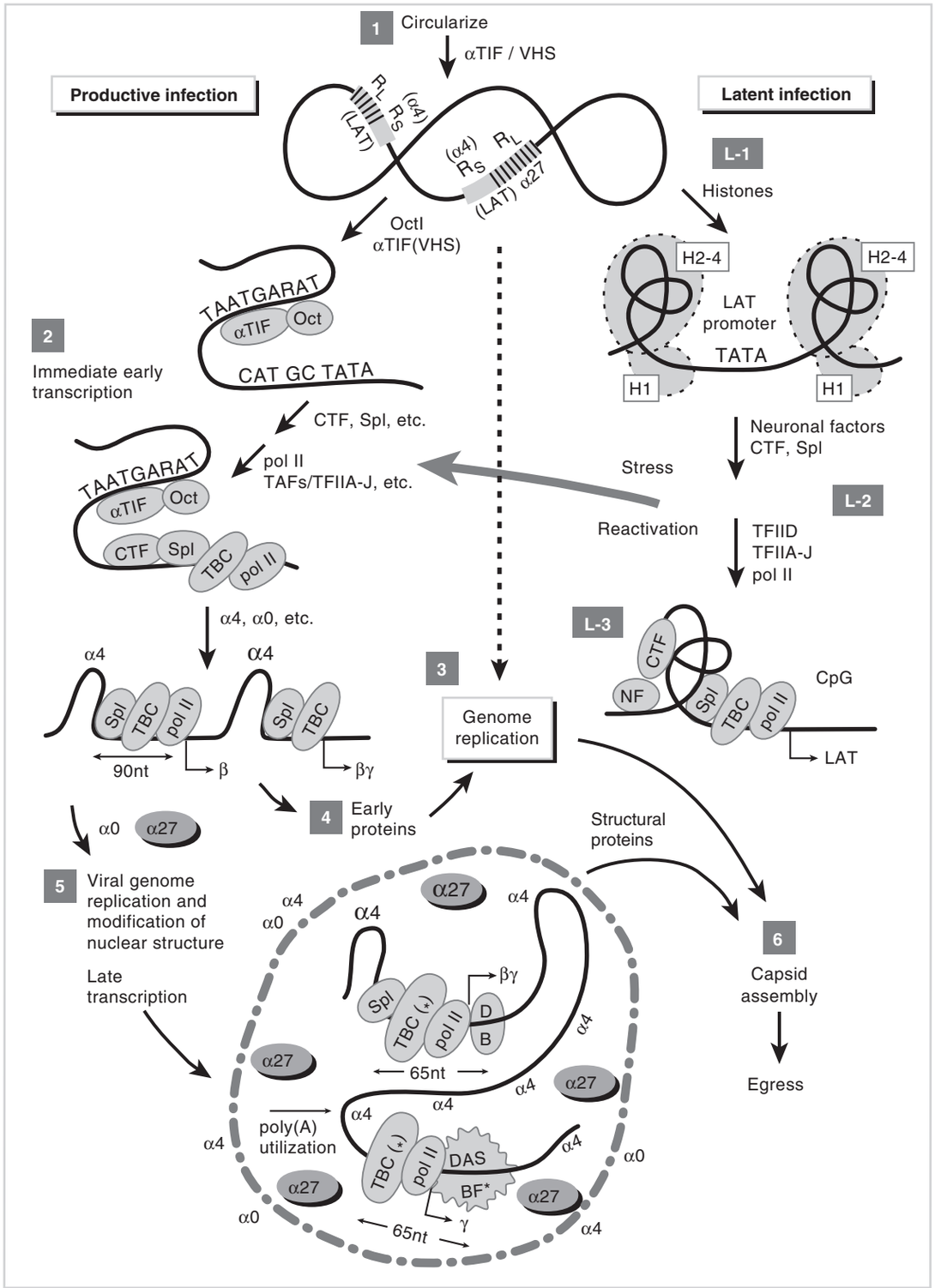

Fig. 18.4 The HSV-1 productive and latent infection cycles. In productive infection, the viral genome becomes circular but does not associate with chromatin proteins (1). This is followed by immediate-early transcription that requires the association of cellular factors (Oct-1) binding to the TAATGARAT sequence element within the immediate-early promoter enhancers and with α-TIF to enhance transcription of immediate-early transcripts (2). These are controlled with promoters with specific enhancers. This process results in transcriptional activation that leads to early transcription, and ultimately, to viral genome replication (3, 4). Viral genome replication is accompanied by rearrangement of nuclear structures and late transcription (5), and this is followed by capsid assembly (6). In latent infection, the earliest transcription does not occur and the viral genome becomes associated with histones to form a mini-chromosome (L-1). This essentially shuts down productive transcription but allows expression of the latency-associated transcript (LAT) (L-2). LAT facilitates the stress-induced reactivation of virus by an unknown mechanism (L-3). Reactivation reinitiates the productive cascade. (vhs, virion-associated host shutoff protein; α-TIF, alpha-trans-inducing factor protein; CTF, SpI, TAFs, TFIID, TFIIA-J, and TBC are all components of eukaryotic transcription machinery as explained in Chapter 13.)

The α47 protein appears to have a role in modulating host response to infection by specifically interfering with the presentation of viral antigens on the surface of infected cells by the MHC class I complex (see Chapter 7).

Early gene expression Activation of the host cell's transcriptional machinery by the action of alpha gene products results in expression of the early or beta genes. Seven of these are necessary and sufficient for viral replication under all conditions: DNA polymerase (U_L30), DNA-binding proteins (U_L42 and U_L29), ori-binding protein (U_L9), and the helicase-primase complex (U_L5, -8, and -52). When sufficient levels of these proteins accumulate within the infected cell, viral DNA replication ensues.

Other early proteins are involved in increasing deoxyribonucleotide pools of the infected cells, while still others appear to function as repair enzymes for the newly synthesized viral genomes. These accessory proteins are "nonessential" for virus replication in that cellular products can substitute for their function in one or another cell type or upon replication of previously quiescent cells. However, disruptions of such genes often have profound effects on viral pathogenesis or viral ability to replicate in specific cells.

Genome replication and late gene expression Viral DNA replication at high levels under the control of virus-encoded enzymes is termed **vegetative DNA replication**. The vegetative replication of HSV DNA occurs in a number of stages that tend to occur simultaneously in the infected cell nucleus. First, HSV-encoded ori-binding and DNA-denaturing proteins bind to one or all of the ori's, and a replication fork carrying out DNA synthesis is generated. This process is shown in Fig. 18.5.

During the replication process, this circular replication structure is "nicked" at a replication fork, and a "rolling circle" intermediate is formed. As shown in Fig. 18.5, such a rolling circle (in theory) generates a continuous concatemeric strand of newly synthesized viral DNA that is available for encapsidation. In actuality, as DNA is being replicated, new synthesis begins at any one of a number of ori's, and highly concatenated, linked networks of DNA are formed in the infected cell. Although these networks are difficult to visualize, and appear as a "tangled mess" in the electron microscope, the packaging process for viral DNA allows individual, genome-sized pieces of viral DNA to be encapsidated.

The encapsidation process involves viral maturation/encapsidation proteins associating with the "a" sequences of the newly synthesized genomes, simultaneously cleaving them from the growing replication complex and packaging them into mature capsids. This process also is shown in Fig. 18.5.

Viral DNA replication represents a critical and central event in the viral replication cycle. High levels of DNA replication irreversibly commit a cell to producing virus, which eventually results in cell destruction. DNA replication also has a major influence on viral gene expression. Early expression is significantly reduced or shut off following the start of DNA replication, while late genes begin to be expressed at high levels.

Immunofluorescence studies using antibodies against specific viral proteins involved in DNA replication and transcription, such as shown in Fig. 18.6, demonstrate that DNA replication and late transcription occur at discrete sites, or "replication compartments," in the nucleus. Prior to DNA replication, the α4 protein and the single-stranded (ss) DNA-binding protein ICP8 (U_L29) are distributed diffusely throughout the nucleus. Concomitant with viral DNA replication, distribution of these proteins changes to a punctate (point-like) pattern. In the case of α4, this change involves interaction with α0 and α27.

Virus assembly and release More than 30 HSV-1 gene products are structural components of the virion and all are expressed with late kinetics. As outlined in Chapter 6, HSV capsids assemble around viral scaffolding proteins in the nucleus, and then other viral proteins interact with

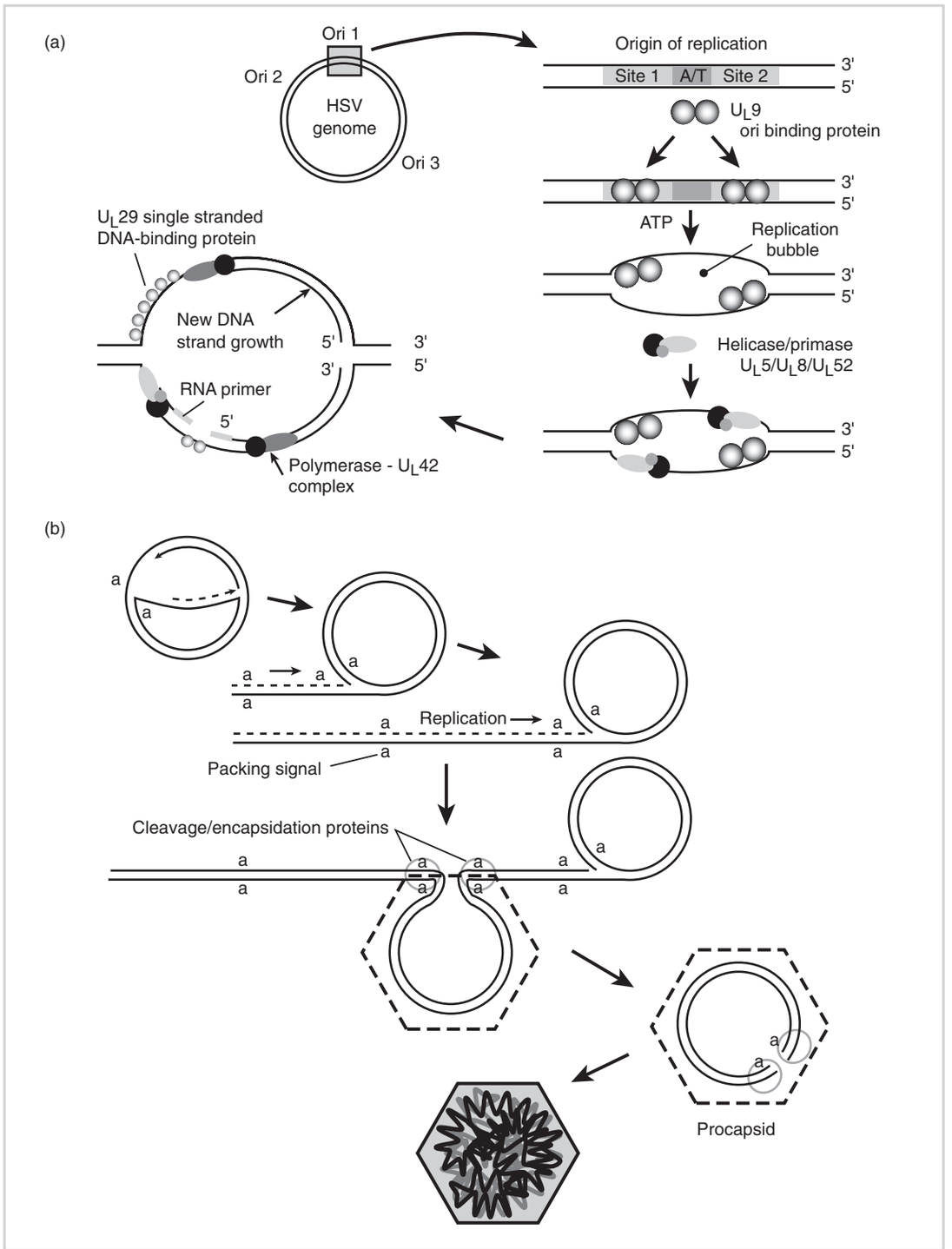

Fig. 18.5 Replication and encapsidation of viral genomes. *a.* HSV DNA initiates rounds of DNA replication at one of three origins of replication (ori). *b.* The genome is circular in the cell, which leads to a structure that is nicked to form a rolling circle. Long concatemeric strands of progeny DNA are encapsidated by the interaction of cleavage/packaging proteins with the specific packaging signals at the end of the viral genomes (the "a" sequences).

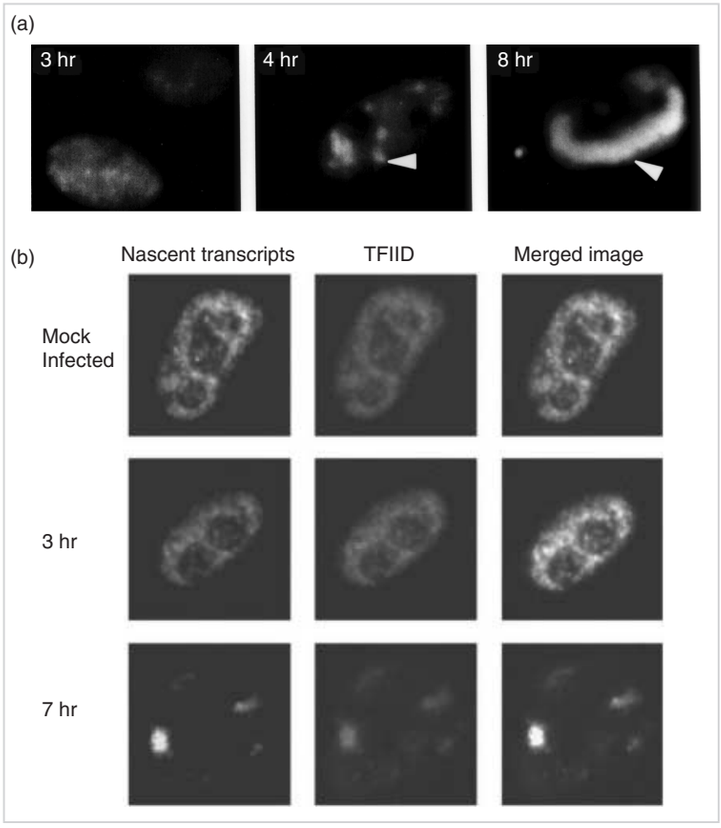

Fig. 18.6 Immune fluorescence analysis of the rearrangement of nuclear structures following HSV-1 infection. *a*. The localization of an antibody to the early single-stranded DNA-binding protein. The antibody is found diffusely distributed in the nucleus of infected cells at 3 hours after infection, but it (and the associated replicating viral DNA molecules) rapidly becomes concentrated in "replication factories" following this. (Photograph courtesy of R. M. Sandri-Goldin.) *b*. Confocal microscopy of infected cell nuclei. In this series of views, an uninfected (mock infected) cell, or a cell at 3 hours or 7 hours following infection was incubated with two antibodies bound to different chromophores. The first (green) is specific for newly synthesized RNA. This trick is accomplished by incubating the infected cells in medium containing a modified nucleotide that is incorporated into RNA. The base (5-Br-uridine) is antigenic, and there is a good commercial antibody available against it. The second (red) is an antibody specific to a component of the pre-initiation complex TATA-associated factor TFIID, and will react with this complex as it forms at the transcription start site. The merged colors (yellow) show that RNA and the transcription complex are confined to localized areas in the infected cell nucleus at the late time. Confocal microscopy is described in Chapter 12. See Plate 8 for a color image.

replicated viral DNA to allow DNA encapsidation. The encapsidated DNA is not associated with histones, but highly basic polyamines (perhaps synthesized with viral enzymes) appear to facilitate the encapsidation process.

Mature capsids bud through the inner nuclear membrane that contains the viral glycoproteins (Fig. 18.7). In the early maturation process in the nucleus, capsids appear to be surrounded by the "primary" tegument protein, U_L31, and this directs the budding through the inner nuclear membrane into which the U_L34 phosphorylated membrane protein has been inserted. These "primarily enveloped" capsids then bud through the outer nuclear membrane where the primary envelope is lost. The cytoplasmic capsids then associate with the numerous tegument proteins of the mature virion, including α-TIF and vhs, which appear to functionally interact to help final envelopment. Final envelopment takes place as the mature capsids and associated tegument proteins bud into exocytotic vesicles, the membranes of which contain all the glycoproteins associated with the mature virions. Infectious virions can either remain cell associated within these vesicles, and spread to uninfected cells via virus-induced fusion, or can be released from the cell in exocytotic vesicles for reinfection such as shown in Fig. 6.9b. Obviously, in the latter case, the virion itself is subject to immune surveillance and host-mediated immune clearance.

Latent infections with herpesviruses

All herpesviruses can establish latent infections in their natural host. Such an infection is characterized by periods of highly restricted (or no) viral gene expression in the cells harboring the latent

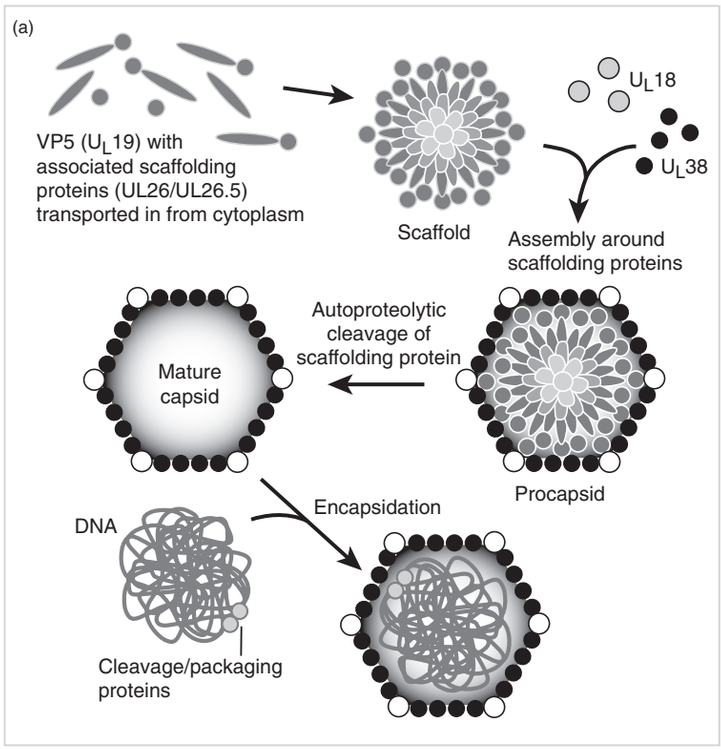

Fig. 18.7 Maturation of the HSV capsid and its envelopment by tegument and virus-modified nuclear membrane. *a.* The procapsid assembles around scaffolding proteins that are then digested away and the empty capsid incorporates DNA by means of the action of cleavage/packaging proteins. *b.* The filled capsid then migrates through the double nuclear membrane by first budding into the intercisternal space between the inner and outer membranes, and then fusing of this initial membrane with the outer nuclear envelope, releasing the capsids into the cytoplasm. Final enveloping is by budding through the walls of the exocytotic vesicle, and final release of the enveloped virion is by fusion of this vesicle with the cytoplasmic membrane as shown in Fig. 6.9.

genomes, interspersed with periods of virus replication and infectivity (reactivation or *recrudescence*). The different types of herpesviruses carry out latency and reactivation processes in generally similar ways, but details depend on the virus in question.

HSV latency and reactivation

Neurotropic herpesviruses like HSV establish a latent infection by entering a sensory nerve axon near the infection site. The virus particle then can migrate to the neuron's nucleus in the nerve ganglion. HSV-1 tends to favor the lip and facial areas for initial infection; hence, the sensory nerve invaded is the trigeminal ganglion. For infections of genital mucosa, HSV-2 invades the sciatic nerve ganglia. If virus gene expression occurs normally, viral DNA replication and cell death will occur; however, in most infected neurons, the earliest stages of gene expression appear to be blocked. Thus, these neurons are, in a sense, nonpermissive for replication. This results from the fact that one or several cellular transcription factors required to express the immediate-early genes are absent or present in a modified form that is different from that found in epithelial and other cells fully permissive for HSV replication.

In the latently infected neuronal nucleus, HSV DNA is present as a histone-wrapped minichromosome or episome, which is in distinct contrast to the viral genome's physical state during productive infection. Since viral DNA is in the neuronal nucleus and fully differentiated neurons do not replicate, the DNA can remain for long periods of time—probably for the host's life. Experimental studies of viral gene expression in neurons during reactivation suggest that when the host is stressed with certain agents such as HMBA (hexamethylene bisacetamide), the cellular transcriptional environment changes, and immediate-early as well as other viral transcripts are expressed at detectable levels. This is sufficient for the replication of virus in at least a few neurons, which results

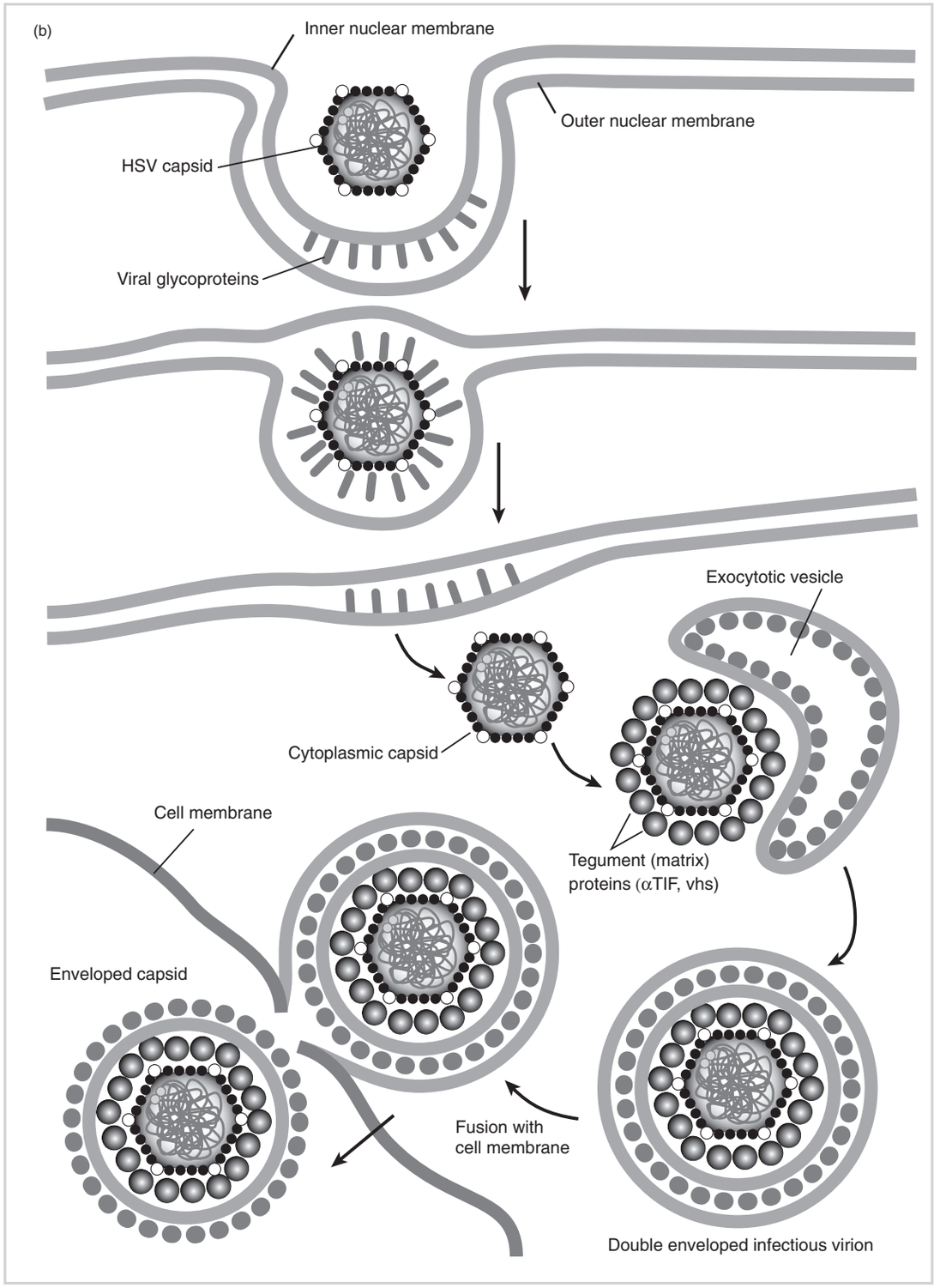

Fig. 18.7 *Continued*

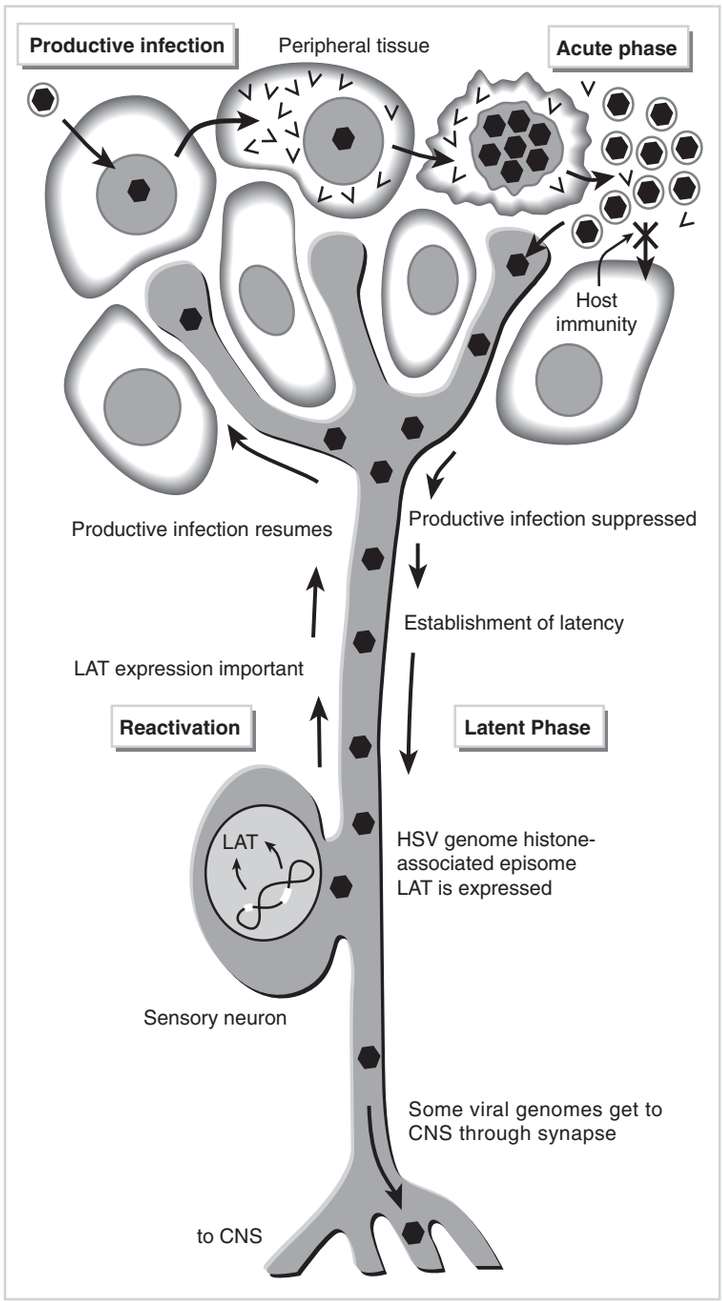

Fig. 18.8 The "decision" made by HSV upon infection of epidermal tissue enervated with sensory neurons. Productive infection follows infection of peripheral tissue, but entry of the virus into neurons leads to latent infection in a significant proportion.

in the transitory appearance of a small amount of virus that travels down the axon and reinfects the area where the virus first infected.

Reactivation can happen many times with no apparent damage to the trigeminal nerve ganglion. It is not known whether one or a few neurons die with each reactivation, but there is such a very large number of neurons that the loss of a few during reactivation does not have a significant effect on enervation of the lip. The process of establishment of and reactivation from latent infection is outlined schematically in Fig. 18.8, and the transcriptional switches involved are indicated in Fig. 18.4.

HSV transcription during latency and reactivation The viral genome of HSV and many (but not all) other alpha-herpesviruses is not fully shut off during latent infection. One transcript family, collectively termed the *latency-associated transcripts* (LATs), is expressed from a single latent-phase active promoter located in both copies of the R_L (see Fig. 18.2). It is not known (yet) just how they work, but they make the reactivation process more efficient so that more virus is produced when the animal is stressed.

The HSV LAT is a large transcript that is weakly expressed during productive infection, but unlike all other known productive cycle transcripts, it does not "shut down" in latently infected cells. This transcript is spliced to generate an unusual 2 kb intron, which — unlike most introns — is stable and accumulates in the infected cell. Indeed, it is this intron that is responsible for the nuclear in situ hybridization signal seen in latently infected neurons. An example of such a signal is shown in Fig. 12.10.

Epinephrine induction of rabbits latently infected with HSV via the ocular route leads to relatively efficient shedding of virus from the eye (see Chapter 3). This induction also results in a transitory expression of productive cycle viral transcripts. This transitory expression can be readily detected using polymerase chain reaction (PCR) amplification of cDNA generated from polyadenylated RNA isolated from rabbit ganglia. An example of such an experiment is shown in Fig. 18.9.

In this experiment, rabbit ganglia were isolated either before induction or at 8 hours after induction. RNA was isolated, and cDNA was synthesized with retrovirus reverse transcriptase from the 3′ end of the RNA using a primer made up of oligodeoxythymidine, which will anneal to the mRNA polyA tail. Then, specific viral cDNA sequences corresponding to those found in ICP4, ICP27, DNA polymerase, and LAT mRNA were amplified with specific primers. The results clearly show the continued presence of LAT mRNA in latent and reactivating ganglia, but the productive-phase transcripts only are seen during a short "window" during reactivation. The simplest interpretation of such an experiment is that the epinephrine changes the transcriptional "program" of a few latently infected neurons that can then produce a small amount of virus. This virus migrates down the nerve axon and establishes a low-level infection in the rabbit cornea, which results in the ability to isolate infectious virus.

Specific HSV genes whose function may be to accommodate reactivation Successful reactivation should not be viewed as merely involving the function of a limited number of genes expressed during the latent phase of infection by HSV. Rather, it is clear that the reactivation process involves a highly orchestrated interaction between a number of viral genes specifically directed toward ensuring efficient replication of small amounts of virus in an immune host.

The earliest events in reactivation of HSV from a latently infected neuron can be envisioned to be similar to those events that might be seen in the expression of viral genes in a cell transfected with infectious HSV DNA. Since transfected DNA does not contain α-TIF, enhancement of immediate-early genes will not take place, but limited expression of early viral genes leads to some viral DNA replication and production of a few infectious virions that can infect neighboring cells. While the process is inefficient, there is no barrier to its occurring in cultured cells, and so plaques will form and virus infection will spread.

However, a similar process in a reactivating host will quickly encounter some profound problems. First, inefficient replication of virus in peripheral cells can induce interferon, which will block further virus replication. Second, the host immune memory will quickly marshal all the immune defenses available to suppress and clear the active replication of virus.

HSV encodes a number of genes to counter these host defenses. First, the inhibition of MHC class I-mediated antigen presentation at the surface of the infected cell by the α47 protein is thought to be effective in slowing the host's ability to detect the earliest stages of productive replication.

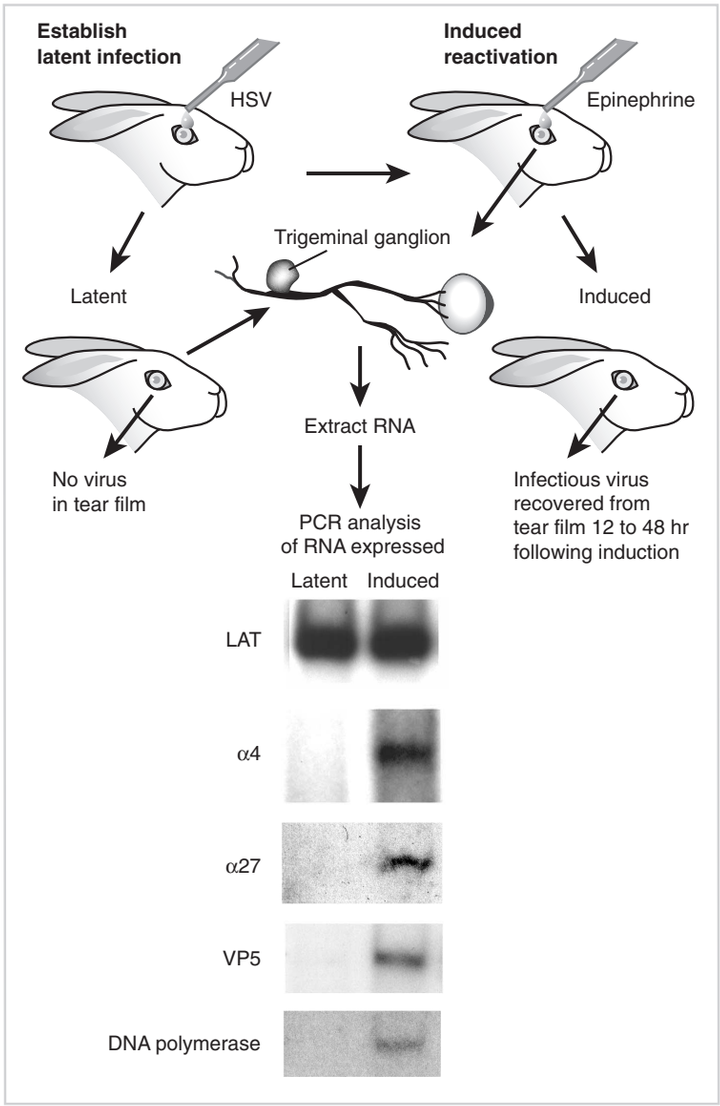

Fig. 18.9 The expression of HSV transcripts during latent infection and reactivation in the rabbit. A standard approach toward experimental investigation, as was briefly described in Chapter 3, is outlined. If a latently infected rabbit is killed and RNA in the trigeminal ganglion extracted and subjected to reverse transcription–polymerase chain reaction (PCR) using primers specific for the latent-phase transcript (LAT), or several productive cycle transcripts (α4, α27, DNA polymerase, or VP5), the only signal seen is with the LAT primer pair. This indicates that only the LAT region is being transcribed with any frequency. Following induction of the rabbit with epinephrine, however, all transcripts are expressed, suggesting that at least some viral genomes are induced to enter productive cycle replication.

Second, HSV encodes a protein, ICP34.5, that specifically blocks interferon's inhibition of translation in the infected cell by inhibiting phosphorylation of the translational initiation factor eIF-2. This has the result of ensuring efficient translation of the small amounts of viral transcripts expressed in this limited infection.

Third, the virus encodes a protein that inhibits the infected cell's tendency to undergo apoptosis, thus ensuring a higher yield of infectious virus.

Other viral proteins also have been identified as having potential roles in interfering with cellular and host defenses, which, if allowed to function, would be very effective in inhibiting replication of the small amount of virus produced by the reactivation step itself.

EBV latent infection, a different set of problems and answers

EBV is a lymphotropic herpesvirus. Although difficult to study in cell culture, its productive replication cycle is generally similar to that outlined for HSV. In the host, the site of primary infection is

apparently the epithelium of the nasopharynx. From this site, the virus infects and establishes a latent infection in normally short-lived circulating B lymphocytes where it cannot replicate because of the lack of essential factors needed to start the productive cascade. In order for the virus to maintain a latent state it must, then ensure that the site of latency is maintained; i.e., it must immortalize the B cells in which its genome resides.

In the lymphocyte, EBV expresses 11 genes that are involved in latency. While this amount of gene expression is considerably greater than that seen with latent infection with HSV, it is still a highly restricted subset of the more than 100 genes encoded by the virus. A number of the latent phase specific genes encode proteins that stimulate the B cell to keep dividing, and to "short-circuit" the genetic controls that the cell contains that would normally induce the death of a cell undergoing unscheduled replication. Other latent genes serve functions analogous to those of the reactivation-directed productive cycle genes expressed by HSV.

Normal circulating B lymphocytes are short-lived. After a period of time, apoptosis is induced, causing the cell to die. As briefly discussed in earlier chapters, the process of apoptosis or programmed cell death is a natural stage in the life cycle of many types of differentiated cells. One benefit of such a process is that cells generated for a specific purpose (such as B lymphocytes) can be eliminated as the need for them decreases. Another benefit is that cells that have undergone a number of replication cycles, and may have accumulated deleterious mutations, can be eliminated.

Both primary and latent infections with EBV are characterized by the expression of a viral gene product interfering with the induction of apoptosis in B lymphocytes. Infected B lymphocytes thus proliferate during initial infection, causing mononucleosis (high levels of monocytes in the blood), which is a bit like leukemia in that white blood cells tend to crowd out red blood cells and cause anemia. EBV-induced mononucleosis is temporary, but a number of EBV-infected B lymphocytes survive as replicating, essentially immortal, reservoirs of latent viral genomes.

The mechanism by which EBV immortalizes B cells appears to involve inactivation of p53 and Rb tumor suppressor genes in a manner roughly similar to that seen in infection with papovaviruses and adenoviruses. Other specific virus-induced processes are also involved, however. One important result of immortalization by EBV is that the chromosomal telomeres are stabilized, perhaps by alteration of the activity of telomerase enzyme in B lymphocytes. This enzyme has an important role in cell mortality (see Chapter 10), and functions to allow the progressive loss of DNA at the ends of chromosomes until a critical point is reached, after which cell death ensues.

While these B lymphocytes are dividing, EBV DNA is present in the cell as an episome just like the situation with papillomavirus infection of basement epithelium. Every time the cell divides, EBV DNA replicates one round, so each daughter cell gets some viral DNA. This replication is under the control of a specific origin: the ori-P (again, like the situation with papillomavirus). Under proper stress, the B lymphocyte can be induced to undergo productive infection. This reactivation also requires some EBV function, and is, again, generally similar to the situation with HSV.

Reactivation of EBV from latent infection requires specific stimulation of the latently infected lymphocyte. This reactivation event faces a number of the same obstacles outlined for HSV reactivation. In response, like HSV, EBV inhibits cell-based interferon defenses against virus infection. In contrast to the mechanism utilized by HSV and analogous to that utilized by adenovirus, however, EBV accomplishes this by the expression of a set of small virus-encoded RNAs, the Epstein-Barr-encoded RNAs (EBERs), which function like adenovirus VA RNAs (see Chapter 17). Like adenovirus VA RNAs, EBERs are expressed via cellular RNA polymerase III.

Pathology of herpesvirus infections

HSV, EBV, and other herpesvirus latent infections are relatively benign conditions, and latency does not appear to cause many serious problems. However, if the host's immune system does not function properly owing to disease or clinically induced immunosuppression (as for organ trans-

plantation), there can be significant medical problems, including disseminated herpesvirus infections in the brain or other tissues. For example, HSV keratitis, which can result in blindness, is a major cause of complications in corneal transplants, leading to blindness.

Primary herpesvirus infection in a newborn (neonate) can be devastating because the infant's immune system has not yet developed fully. It also is possible that chronic HSV reactivation has a role in the activation of chronic low-level infections with HIV, resulting in the development of AIDS.

Herpesviruses as infectious co-carcinogens

In areas of the world where there are many cases of malaria, EBV coinfection with a malaria infection can lead to a type of cancer called *Burkitt's lymphoma*. This cancer is found in portions of tropical Africa. In Japan and China, eating some very complex hydrocarbons found in foods pickled by fungal fermentation can interact with EBV-infected cells to lead to *nasopharyngeal carcinoma*. Both types of cancers are, then, associated with EBV infection, but require a **co-carcinogen** for development. This is probably true for papillomavirus-caused cancers, and is also true for hepatitis B virus–associated liver carcinomas.

The co-carcinogen functions in some way to induce mutations in cells that are persistently dividing due to the presence of EBV latent gene products. These mutations eventually lead, as is the case with papillomavirus carcinogenesis, to metastasizing cancer cells. Again, like papillomavirus carcinogenesis, the viral genome becomes integrated into the cellular genome during the process of cell transformation. Following this, no further expression of viral genes is necessary for maintenance of the viral genome. However, unlike papillomavirus integration, EBV DNA integration tends to be at a specific chromosomal site.

A second human herpesvirus, HHV-8, is also associated with a human cancer called *Kaposi's sarcoma* (KS). This cancer was first described in the last part of the nineteenth century as a rare disease of very old men. It is marked by a slow progression of the formation of sarcomas made up of highly pigmented epithelial cells, is not particularly invasive, but eventually leads to death. Its occurrence in the general population is known to be associated with extensive loss of cellular immune capacity and specific geographical and genetic factors.

A high incidence of KS was observed beginning in the early 1980s in homosexual men in a number of gay communities, notably San Francisco, New York, and Los Angeles. Victims were found to have advanced immunodeficiency, and the study of this growing epidemic led to the discovery of HIV as a cause of AIDS.

Despite the high frequency of KS in some gay communities in which AIDS was common, a number of factors demonstrate that the decline in immunity in the late stages of HIV-induced disease is not the sole causal factor in this cancer. The most obvious one is that many groups of HIV-positive individuals go on to exhibit the symptoms of AIDS without any development of KS. Epidemiological analysis strongly suggests the action of a co-carcinogen working along with HIV in development of the disease.

Recently, HHV-8 was found to be present in 70% to 80% of individuals at risk for development of KS while it is present in less than 2% of the general population. Further, HHV-8 DNA is readily found in sarcoma tissue of AIDS patients, while it is difficult, if not impossible, to find it in other tissues of the same individual.

This finding, along with the known ability of EBV to act as a co-carcinogen in the formation of Burkitt's lymphoma and nasopharyngeal carcinomas, suggests the strong possibility that HHV-8 infection, along with HIV-induced loss of immune capacity, is a contributing factor in the development of KS. Consistent with this possibility is the fact that HHV-8 encodes a number of cell-derived genes known to function in the oncogenic transformation of specific cells. These include the *bcl-2* gene, which inhibits apoptosis; a G protein-coupled receptor, which is active and can

cause transformation of cultured cells; and a gene related to cellular K-cyclin, which can induce contact inhibited cells to enter the S phase and begin the process of division.

BACULOVIRUS, AN INSECT VIRUS WITH IMPORTANT PRACTICAL USES IN MOLECULAR BIOLOGY

Arthropods are infected by a wide variety of viruses. Some of them, the classic arboviruses such as yellow fever virus, dengue virus, and La Crosse encephalitis virus, replicate within the cells of their arthropod hosts but cause no cytopathology and no disease. Other viruses infect their arthropod hosts and cause significant pathology and disease. Among these are the baculoviruses, grouped into the nuclear polyhedrosis viruses (NPVs) and the granulosis viruses (GVs). Some baculoviruses infect insects such as the alfalfa looper (*Autographa californica*), while others infect arthropods such as the pinaeid shrimp. The virus infecting the alfalfa looper (AcNPV) has been extensively studied because of its value in biotechnology (discussed in a following section).

Virion structure

Baculoviruses are large and complex in structure. The genome is large (80–230 kbp), circular, double-stranded (ds) DNA contained in a nucleoprotein core within a capsid. The rod-shaped capsid is composed of ring-shaped subunits that are 30 to 60 nm in diameter and stacked longitudinally to give an overall length of 250 to 300 nm.

Virions can be found in two forms. Budded viruses (BVs) have acquired a virus-modified envelope to surround the capsid as they exit the infected cell through the plasma membrane. This form of the virus is involved in secondary infection from the initial site of entry into the insect.

Occluded viruses (OVs) have an envelope that appears to be derived from virus-modified nuclear membrane. This form of the virus is found embedded within a matrix consisting of a crystalline lattice of a single protein, polyhedrin for NPVs and granulin for GVs. NPVs often exhibit several nucleocapsids embedded in this matrix; however, only a single capsid is embedded in the OV matrix, resulting from GV infections.

The OV form is transmitted horizontally during feeding by the insects. The very stable matrix serves to protect the virions from the environment, but readily dissolves in the insect's midgut, with its high pH, just prior to infection of cells at that site.

Viral gene expression and genome replication

Viral gene expression and genome replication take place in the cell's nucleus. The genome size of these viruses predicts a large coding capacity, and indeed, there are well over 100 viral proteins expressed.

The infectious cycle is divided into early and late phases, followed by the occlusion phase. During the early phase, viral mRNAs are transcribed by the host's RNA polymerase II. Normal post-transcriptional modifications take place to viral mRNAs, although only one transcript is RNA spliced. As would be expected, some of the proteins encoded by these early genes are required for replication of the viral DNA.

Late gene expression occurs following the onset of viral DNA replication. This switch in transcription is mediated by a virus-encoded RNA polymerase that recognizes a unique set of promoter sequences. The encoding of a unique viral RNA polymerase is unusual for eukaryotic DNA viruses, but is seen in the replication of cytoplasmic DNA viruses and in many DNA-containing bacteriophages. These are discussed in Chapter 19.

Polyhedrin protein is synthesized very late in the replication cycle, and it is during this stage that the occluded form of the virus begins to accumulate. The production of polyhedrin is not required for viral replication and the protein gene can be deleted without affecting production of progeny virus. This property has been exploited as a useful tool for applications in biotechnology as discussed in a subsequent section.

Pathogenesis

Some baculoviruses infect insect pests, but some infect ecologically and economically important insects, including silkworms. Infection of a susceptible insect by a virulent virus such as AcNPV leads to a distinctive cytopathology and ultimately to death. Larvae of the leafhopper infected with AcMNPV eventually have viral replication carried out in virtually every tissue. This results in the larvae disintegrating into a liquid that consists of mostly virus particles within their polyhedron matrices. This phenomenon is called *melting* and is essentially macroscopic lysis.

Importance of baculoviruses in biotechnology

Because of their high infectivity for insects, and because they display narrow host ranges, baculoviruses have become important tools in the battle against pests that feed on important plant species. Many researchers hope that baculovirus can replace chemical pesticides as a biological control agent, for instance, for control of the apple maggot in Europe, the coconut beetle in the South Pacific, and soy bean pests in Brazil. There is hope that baculoviruses may also be effective in controlling the tussock moth larvae in the forests of the Pacific Northwest.

A second role for these agents is in the laboratory. Since polyhedrin protein is not essential for viral replication, the gene encoding it can be deleted from viral DNA with no effect on virus replication. Also, the promoter controlling expression of the polyhedrin gene is quite active. Accordingly, deletion of the coding region of the polyhedrin gene allows the insertion of foreign genes of reasonably large size whose expression is under control of the baculovirus promoter. Thus, infecting insect cells in culture allows expression of the cloned gene in an invertebrate cell that processes the protein in much the same way as a vertebrate cell. This system is useful for producing large amounts of correctly modified and folded eukaryotic gene products.

QUESTIONS FOR CHAPTER 18

1 What feature in herpes simplex virus type 1 (HSV-1) allows the virus to evade the immune system and establish a latent infection?

2 HSV does not alter tumor suppressor gene products. Considering this, how does HSV get around the fact that the host cell is not transcriptionally active?

3 You have isolated the viral DNA of two separate cell cultures infected with virus. You know that one viral infection is due to the SV40 while the other is due to HSV. In each case, the DNA was isolated at a time exhibiting a great deal of cytopathology. Further, the DNA you have isolated from one of the cultures contains almost no single-stranded material. In order to determine which culture was infected with which virus, you can do one or more of the following:

a Check the other culture. It should have a lot of single-stranded DNA and would be the one infected with HSV.

b Measure the density of DNA isolated from the cultures. If one has a significant amount with a density indicative of a high G + C content, that is the one infected with HSV.

c Take total DNA from the infected cultures and gently sediment it. Try to isolate relatively small circular DNA molecules, which would be indicative of SV40 infection.

Continued

d Isolate the DNA from the infected cells and digest with *Eco*RI restriction enzyme and do a Southern blot on the digest. The culture infected with SV40 should yield a fragment about 5400 bp, which will hybridize to SV40 DNA probe.

Which of these methods would *not* give you the information you need?

4 HSV is a member of the Herpesviridae family. The virus enters the cell by membrane fusion and the dsDNA genome is transported into the nucleus of the cell where viral gene expression begins.

a Delivered to the nucleus, along with the DNA genome, is the viral DNA-binding protein α-TIF. What is the function of this protein?

b The protein α-TIF is actually made late during viral replication (it is one of the gamma class of genes). What will be the effect on herpesvirus gene expression if a cell is infected with a temperature-sensitive α-TIF mutant and the cells are placed at 39.5°C (non-permissive temperature) early during infection (within the first hour)? Late during infection (after 16 hours)?

5 Briefly explain why the herpesvirus α-TIF protein is the product of a late (gamma) gene whose action is required early during infection.

6 The drug acyclovir is a guanosine analogue that is a specific antiviral agent for certain members of the family Herpesviridae. When HSV-1 is grown in cell culture in the presence of this drug, acyclovir-resistant mutants of HSV-1 can be selected. Name two HSV-1 genes that can be mutated to make the virus acyclovir resistant and give a brief reason for the resistance in each case.

7 HSV-1 can infect epithelial cells and then go on to establish a latent infection in the basal ganglia. In the table below, indicate (with a "Yes" or a "No") which viral feature will be found in each kind of cell. Assume that the basal ganglia cells are in the latent state and have not yet been reactivated.

HSV-1 Feature	Epithelial cells	Basal ganglia cells
Viral DNA in the nucleus		
Expression of the alpha class of viral transcripts		
Expression of LAT1 RNA		
Production of viral capsid proteins		