Chapter 13
Herpesvirus Genome Replication

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The Herpesviruses

The Herpesviridae are a large family of enveloped, double-stranded DNA viruses that are responsible for many human and veterinary diseases. Herpesviruses can infect mammals, birds, and reptiles, and so far, eight distinct family members have been found which infect humans including herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2), Epstein–Barr virus (EBV), cytomegalovirus (HCMV), varicella-zoster virus (VZV), human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7), and Kaposi's sarcoma herpesvirus (KSHV). All members of the family are capable of both lytic and latent infections although they differ greatly in tissue tropism and in many aspects of their interactions with their hosts. These viruses share many aspects of virion structure (they all are T = 16), genomic organization, mechanisms of DNA replication and life cycle. Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) are the most extensively studied of all the herpesviruses, in part because they are most amenable to genetic and biochemical approaches. This chapter will focus primarily on herpes simplex virus type 1 (HSV-1); however, other human herpesviruses will be discussed when their replication strategy differs in significant detail from simplex viruses.

Overall Virus Life Cycle

HSV initiates viral infection by specific binding of viral glycoproteins to cell surface glycosaminoglycans and cellular receptors (Spear 2004). Following entry, capsids have been shown to translocate along microtubules (Sodeik et al. 1997) to the nuclear pores where they dock and presumably eject their genomes into the nucleus (Ojala et al. 2000). A tightly regulated cascade of gene expression occurs

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consisting of three well-defined kinetic classes of genes: immediate early, early, and late. Viral gene expression, DNA replication, and encapsidation occur within globular domains in the nucleus termed replication compartments (Knipe 1989; Lamberti and Weller 1996). Herpesvirus genomes replicate in the nucleus through the formation of longer-than-unit length concatemers. Monomeric units are cleaved from concatemers during a packaging reaction which occurs in conjunction with the uptake of genomes into preassembled capsids. DNA-containing capsids exit the nucleus by budding through the nuclear membrane, and they acquire their final envelope and mature glycoproteins by a series of envelopment and de-envelopment steps (reviewed in [Baines and Weller 2004]). Unlike other enveloped viruses, herpesviruses are known to package approximately 20–30 proteins between the capsid and the envelope in a region termed the tegument or "skin". Many tegument proteins are known to play active roles in the earliest stages of infection as they are brought in with infecting virions into newly infected cells and play regulatory roles in shut off of host protein synthesis and the stimulation of immediate-early viral gene expression.

Herpesvirus Genomic Structure

Herpesvirus genomes vary in size from 125 to almost 250 kbp and contain both unique and repeated regions. Most herpesviruses contain direct terminal repeats and many also contain repeated sequences in an inverse orientation internally. The HSV genome (152 kbp) contains two unique regions (U_L and U_S) flanked by inverted repeat sequences (Hayward et al. 1975). The *a* sequence is present in three locations in the viral genome: at both termini and an inverted copy is present at the U_L–U_S junction; the *b* sequences flank the U_L segment and the *c* sequences flank U_S (Fig. 13.1A). The HSV genome undergoes genomic inversions in which the unique regions, U_L and U_S, invert with respect to one another during replication. Inversion events may be related to the propensity of HSV to undergo recombination described below.

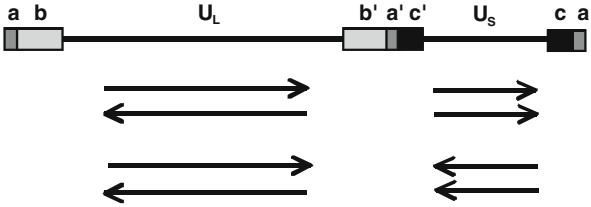

Fig. 13.1 HSV genome. The HSV-1 genome consists of two unique regions UL and US flanked by repeated sequences. UL is flanked by *ab* and *b'a'*, and US is flanked by *a'c'* and *ca*. During infection, the two unique regions invert relative to each other. The arrows reflect possible orientations of the UL and US segments as a result of genomic inversion.

Gene Expression and Regulation

HSV-1 is believed to encode over 80 open reading frames, and gene expression is very tightly temporally controlled. Viral genes are classified as immediate early, early, or late and are transcribed from both strands of the viral genome. Most of the immediate-early genes encode regulatory proteins while the early gene products are primarily involved in viral DNA replication. Many of the structural proteins are encoded as late genes which are not expressed until viral DNA synthesis has occurred.

Cis- and *Trans*-Acting DNA Replication Factors

Origins of Replication

Three origins of replication have been mapped on the HSV-1 genome: two copies of OriS within the repeated *c* region and one copy of OriL within the unique long region (reviewed in [Challberg 1996]). Both origins contain binding sites for the origin-recognition protein, UL9. OriS consists of a 45-bp palindrome containing an A/T-rich region flanked by two recognition sites for UL9 (box I and box II). A third weaker binding site (box III) is located to the left of box 1 outside the palindromic region (Fig. 13.2A). OriL consists of a longer perfect palindrome, 144 bp in length, that contains four recognition sites for UL9 (two copies of box I and two copies if box III) (Fig. 13.2B). Both oriL and oriS are located in the promoter-regulatory regions of divergently transcribed genes: oriS is positioned between two immediate-early genes, ICP4 and ICP22/27, while oriL is located between two early genes, ICP8 (UL29) and the catalytic subunit of the polymerase (UL30). OriL and one copy of oriS can be deleted without affecting the ability of the virus to multiply, suggesting that viral replication can occur in genomes containing only one copy of the origin (Igarashi et al. 1993; Polvino-Bodnar et al. 1987). The origins of replication of other alpha-herpesviruses are similar to those of HSV-1 and HSV-2; however, the origins of replication of the beta- and gamma-herpesviruses are much longer and more complex (Anders and McCue 1996; Yates 1996).

Trans-*Acting HSV-1 Replication Proteins*

The HSV-1 genome encodes seven essential replication proteins and several non-essential replication proteins. The seven essential replication proteins include a single-strand DNA-binding protein (known as ICP8 or UL29), a two subunit DNA polymerase (UL30 and UL42), a three subunit helicase/primase complex (UL5, UL8, and UL52), and an origin-binding protein UL9 (reviewed in [Chattopadhyay et al. 2006; Marintcheva and Weller 2001a; Weller and Coen 2006]). Interestingly, homologs of the first six of these are also encoded by all other human and animal

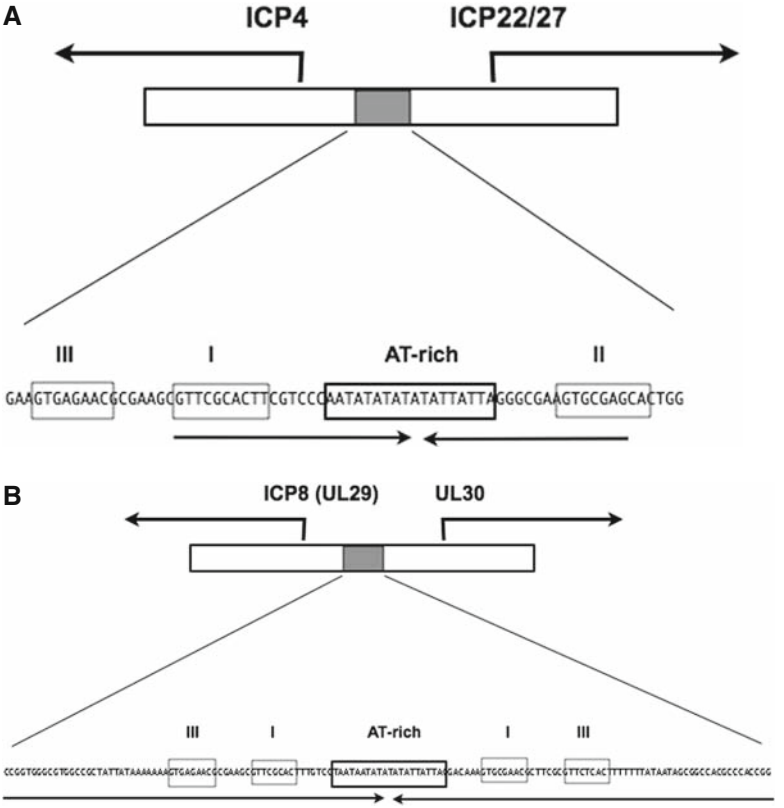

Fig. 13.2 Origins of DNA replication. **A**. OriS can be depicted with three recognition sites for UL9, the origin-binding protein (boxes I, II and III, marked in *gray*) and an AT-rich linker (marked in *black*) positioned between boxes I and II. OriS is positioned between two immediate-early transcripts. **B**. OriL is positioned between two divergently transcribed early mRNAs. Recognition sites for UL9 are boxed in *gray*, and the AT-rich regions are boxed in *black*. Since OriL is a perfect palindrome, the recognition sequences are designated I and III on each side of the palindrome.

herpesviruses and are considered to function as the "core" replication proteins with all the necessary enzymatic activities to stimulate DNA replication on a primed in vitro replication substrate. The conservation of functions suggests that the overall strategy of lytic viral DNA replication is conserved within this family. The mechanism and regulation of initiation of viral DNA synthesis, however, is probably shared only by the alpha-herpesviruses. As mentioned above, the origins of replication for beta- and gamma-herpesviruses are more complex, and in addition, no clear UL9 homologs have been identified.

In addition to the six core replication proteins and the origin-recognition protein, HSV encodes a number of proteins which are not essential for viral DNA synthesis including enzymes involved in nucleotide biosynthesis and DNA metabolism such as thymidine kinase, ribonucleotide reductase, uracil–DNA–glycosylase,

Table 13.1 Auxiliary HSV DNA replication genes

Gene	Alternate abbreviation	Major function	Essential for DNA replication in cultured cells?
UL23	TK	Thymidine kinase	No
UL39	RR1	Large subunit of ribonucleotide reductase	No
UL40	RR2	Small subunit of ribonucleotide reductase	No
UL2	UNG	Uracil-DNA glycosylase	No
UL50	dUTPase	Deoxyuridine triphosphatase	No
UL12	Alkaline exonuclease	Putative viral recombinase subunit	No

deoxyuridine triphosphatase, and the alkaline nuclease (Table 13.1) (reviewed in [Weller and Coen 2006]). Ribonucleotide reductase consists of two subunits, RR1 and RR2, and both are needed for enzymatic activity whose function is to produce dNTPs used in DNA synthesis. Recent reports raise the interesting possibility that the HSV RR1 subunit may also act as a chaperone perhaps to prevent the induction of apoptosis and/or to promote the assembly of the translational machinery (Chabaud et al. 2003; Langelier et al. 2002; Perkins et al. 2002; Walsh and Mohr 2006). The deoxyuridine triphosphatase (dUTPase) and uracil–DNA–glycosylase may be important in preventing misincorporation of uracil residues into the viral genome. The HSV alkaline nuclease (UL12) is a 5′ to 3′ exonuclease; in combination with ICP8, UL12 is capable of a strand exchange activity in vitro, and these two proteins may play a role in single-strand annealing (SSA) during infection (Reuven et al. 2004a, 2003) (see below).

HSV-1 Origin-Binding Protein, UL9 (94 kDa)

Genetic analysis indicates that the origin-binding protein UL9 is essential for viral DNA replication (Carmichael et al. 1988). The analysis of temperature-sensitive (*ts*) mutants indicates, however, that UL9 is required early in HSV-1 infection but not late in infection, once DNA synthesis has initiated (Schildgen et al. 2005). Activities associated with UL9 include nucleoside triphosphatase, DNA helicase on partially double stranded substrates, ability to form dimers in solution and ability to bind cooperatively to viral origins (reviewed in (Chattopadhyay et al. 2006; Weller & Coen 2006)). The seven conserved helicase motif characteristic of the SF2 family of helicases reside in the N-terminal domain (residues 1–534) while the domain responsible for specific origin binding has been mapped to the C-terminal one-third of UL9 (residues 564–832) (reviewed in [Chattopadhyay et al. 2006]). Genetic analysis demonstrated that the conserved helicase motifs are essential for both the

in vivo and the in vitro ATPase and helicase activities of UL9 (Malik and Weller 1996; Marintcheva and Weller 2003a, b, 2001b). UL9 has been reported to interact with several other viral proteins including ICP8, UL8 (a component of the trimeric helicase/primase), and UL42 (the DNA polymerase accessory protein) (reviewed in [Weller and Coen 2006]).

UL30/UL42

HSV DNA polymerase comprises a catalytic subunit (UL30) and an accessory subunit which stimulates processivity (UL42) (Anders and McCue 1996). The catalytic subunit, UL30, is a member of the alpha-like DNA polymerase family and possesses a 3′–5′ exonuclease activity. Thus, UL30 contains motifs conserved in other polymerases, and mutations in these motifs have been shown to affect binding of dNTPs and/or their incorporation (Huang et al. 1999). The crystal structure of UL30 has been solved confirming that this protein resembles other alpha-like DNA polymerases (Liu et al. 2006).

The processivity subunit UL42 functions by an unusual mechanism. While other processivity subunits such as PCNA operate as "sliding clamps", which form multimeric rings in solution and are loaded onto DNA with the aid of clamp loaders, UL42 binds to DNA by itself as a monomer with relatively high affinity (Randell and Coen 2004; Weisshart et al. 1999). UL42 can diffuse linearly (slide) on DNA despite its high affinity for DNA (Randell and Coen 2001) and has recently been shown to affect replication fidelity (Jiang et al. 2007). Interestingly, the crystal structure of UL42 suggests that it shares a similar overall structural fold with the processivity factors which function as sliding clamps (Zuccola et al. 2000).

UL5/UL8/UL52

The HSV-1 helicase/primase is a heterotrimer consisting of the products of the UL5, UL8, and UL52 genes, and genetic data indicate that all three are essential for viral DNA replication in cell culture (reviewed in [Marintcheva and Weller 2001a; Weller and Coen 2006]). The HSV-1 UL5/8/52 complex exhibits DNA-dependent ATPase, primase, and helicase activities (reviewed in [Chattopadhyay et al. 2006; Weller and Coen 2006]). UL5 and UL52 together possess all known enzymatic activities while UL8 appears to play a stimulatory role in addition to being able to interact with other members of the replication machinery including UL9, HSV Pol, and ICP8 (reviewed in [Chattopadhyay et al. 2006; Weller and Coen 2006]). The sequence of UL5 reveals seven motifs found in a large helicase superfamily, SF1, and genetic analysis indicates that these motifs are essential for viral DNA replication (Zhu and Weller 1992) and for helicase and ATPase activities in vitro (Graves-Woodward et al. 1997; Graves-Woodward and Weller 1996). UL52 contains a DXD signature motif conserved in many primases, and this motif is essential for viral DNA

replication and for primase activity in vitro (Dracheva et al. 1995; Klinedinst and Challberg 1994). The C-terminus of HSV-1 UL52 contains a putative zinc-binding motif, which is also present in prokaryotic and eukaryotic primases (Carrington-Lawrence and Weller 2003; Chen et al. 2005, 2007). The presence of these motifs suggests that the helicase and primase activities of the complex likely reside in the UL5 and UL52 subunits, respectively; however, several lines of evidence suggest a more complex interaction between these two subunits. Mutations in the putative zinc finger at the C-terminus of UL52 have been shown to abrogate not only primase but also ATPase, helicase, and DNA-binding activities of the UL5/UL52 subcomplex (Biswas and Weller 1999, 2001; Chen et al. 2005). These results suggest that UL52 binding to DNA via the zinc finger may be necessary for loading UL5 onto DNA. Alternatively, it is possible that UL5 and UL52 share a DNA-binding site created by interaction between the two subunits.

UL29 or ICP8

ICP8, encoded by the UL29 gene, is the HSV major single-strand DNA-binding protein and is essential for DNA replication. ICP8 (SSB) is a 130-kDa zinc metalloprotein that preferentially binds ssDNA in a non-sequence specific and cooperative manner. ICP8 exhibits helix-destabilizing activities which are thought to play a role in unwinding duplex DNA during DNA synthesis. ICP8 interacts with many viral and cellular proteins (reviewed in [Challberg 1996]). In particular, ICP8 has been reported to enhance biochemical activities of UL9, the helicase/primase, and polymerase ([Arana et al. 2001; Boehmer 1998; Hamatake et al. 1997] and refs therein). ICP8 also interacts with the viral nuclease UL12 and together these proteins exhibit a strand-annealing reaction ([Reuven and Weller 2005] and refs therein). ICP8 has also been reported to regulate viral gene expression by repressing transcription from the parental genome and stimulating late gene expression from progeny genomes ([McNamee et al. 2000] and refs therein).

The structure of ICP8 lacking its C-terminal 60 residues was recently solved, revealing two separate domains, the N- (aa 9–1038) and the C- (aa 1049–1129) terminal domain, connected by a short linker region of loose electron density (Mapelli et al. 2005). The N-terminal domain forms three regions: the head, neck, and shoulder which in turn are formed from non-contiguous secondary structural elements.

Overview of HSV DNA Replication

Although several *cis*- and *trans*-acting elements have been shown to be required for HSV DNA replication as described above, very little is known about the actual mechanisms of HSV DNA replication. The model presented below is for the most part consistent with existing data; however, validation will require additional experimental support.

Fate of Incoming Viral DNA

HSV-1 DNA genomes enter the nucleus following docking of the capsid at a nuclear pore. Once the linear viral DNA is released it is thought that these linear DNA molecules lose their free ends by a process that does not require de novo protein synthesis (Poffenberger and Roizman 1985). The simplest interpretation of these results is that the viral genome loses its free ends through the formation of a covalently closed circular molecule leading to the model that linear virion DNA is rapidly circularized in infected cells. Aspects of this model have recently been challenged experimentally (Jackson and DeLuca 2003), and considerable controversy still surrounds the fate of the incoming viral DNA soon after infection (Strang and Stow 2005). It is possible that the viral genome adopts an endless configuration by an intra- or intermolecular homologous recombination event; however, additional experimentation will be needed to determine the precise fate of the incoming viral genome.

Initiation of Viral DNA Replication

Current models suggest that the origin-recognition protein, UL9, binds one or more of the origins of replication and recruits the rest of the replication machinery to the origin, stimulating DNA synthesis. Although DNA synthesis is probably initiated by a UL9-dependent step, replication at the origins of HSV alone would not be sufficient to generate the observed head-to-tail concatemers; therefore, we have proposed that HSV DNA replication occurs in two stages (Fig. 13.3) (Weller and Coen 2006; Wilkinson and Weller 2003). We have proposed that UL9 is essential during the first stage; however, later in infection DNA replication appears to proceed in an origin-independent manner (Blumel et al. 2000; Blumel and Matz 1995), which may proceed by rolling circle replication and/or a recombination-dependent replication step (Wilkinson and Weller 2003). The involvement of recombination would be analogous to the replication program of the bacteriophages T4, lambda, and many other linear dsDNA bacteriophage.

The precise role of UL9 in the initiation of HSV DNA replication remains uncertain. Although UL9 is known to possess ATPase and helicase activities in addition to its origin-binding properties, it is unable to unwind blunt-ended linear or circular double-stranded DNA containing an origin of replication. The failure of UL9 to unwind duplex origin DNA remains a major impediment to the establishment of an in vitro DNA replication system. Although the details are not yet known, it is believed that UL9 acts in conjunction with ICP8 to distort or perturb the region, followed by the recruitment of the HSV-1 helicase/primase (H/P) complex (Fig. 13.4). We have demonstrated that an active primase is needed to recruit the HSV DNA polymerase to viral foci in infected cells. Once the HSV DNA polymerase is recruited to the fork, it is believed to be responsible for both leading and lagging strand DNA syntheses. The first stage of DNA synthesis may be bidirectional proceeding from one or more of the three origins of replication. The end products of DNA replication are longer-than-unit-length head-to-tail DNA concatemers.

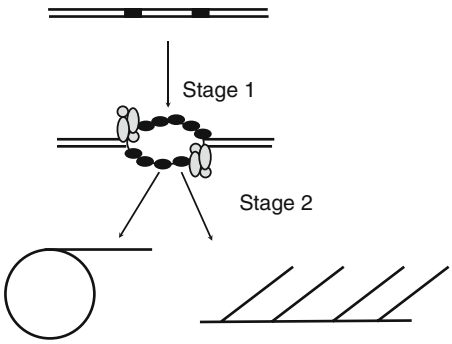

Fig. 13.3 Model for UL9-dependent and UL9-independent HSV DNA replication. According to this model the first stage of HSV DNA replication involves origin unwinding and bidirectional DNA replication (*Stage I*). In this step, UL9 likely acts in conjunction with ICP8. Following the opening, the helicase/primase can be recruited to the complex followed by either recombination-dependent or rolling circle replication (or both) (*Stage II*). Light gray symbols represent UL9 and dark gray symbols represent ICP8, the single-strand DNA-binding protein.

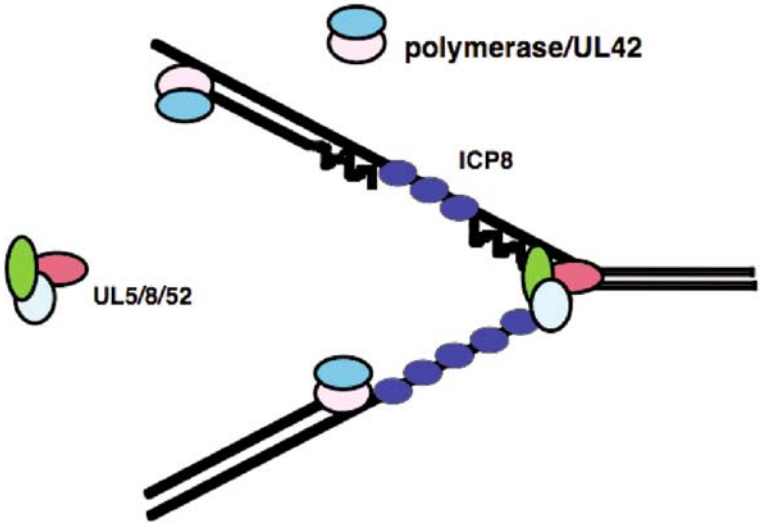

Fig. 13.4 HSV-1 replication fork. An HSV-1 replication fork would be expected to contain the helicase/primase complex (UL5/UL52/UL8) at the fork: UL5 would be expected to unwind duplex DNA ahead of the fork and UL52 would be expected to lay down RNA primers which could then be extended by the two subunit DNA polymerase (UL30 and UL42). The HSV-1 pol would also be expected to carry out leading strand synthesis. ICP8 (UL29, SSB) would be expected to bind to ssDNA generated during HSV DNA synthesis.

Replication Intermediates Are Complex and Branched

Although direct evidence is lacking, several lines of evidence suggest that recombination-dependent replication occurs during the second stage of viral DNA synthesis. (i) We and others have shown that replication intermediates in HSV-1-infected cells are present in a non-linear structure which cannot enter a pulsed field gel, even after digestion with a restriction enzyme which recognizes a single-restriction site within the HSV genome (reviewed in [Wilkinson and Weller 2003]). This complex, perhaps branched, structure is consistent with recombination-dependent replication. (ii) Inversion of the unique regions of the HSV genome has occurred at the earliest times that replicated DNA can be detected (Lamberti and Weller 1996). (iii) Severini et al. (1996) isolated DNA from the well of a pulsed field gel, and following restriction enzyme digestion, fragments were subjected to two-dimensional gel electrophoresis; both Y-shaped arches and X-shaped junctions were observed (Severini et al. 1996). (iv) It has been shown that SV40 DNA replicates by theta replication, resulting in interlocked circles. SV40 DNA replicated by the HSV-1 core replication machinery in infected cells, however, adopts a complex-branched DNA indistinguishable from that of replicating HSV DNA (Blumel et al. 2000). These results taken together suggest that replicating DNA adopts a complex, most likely branched, structure consistent with the involvement of recombination-dependent replication.

The demonstration that HSV encodes a two subunit complex consisting of ICP8 and UL12 which can perform strand exchange is also consistent with a recombination-dependent replication mechanism of DNA replication. This two subunit complex may play a role analogous to that of the red recombinase system encoded by bacteriophage lambda (Reuven et al. 2003; Reuven and Weller 2005; Reuven et al. 2004b). Furthermore, ICP8 has been reported to interact either directly or indirectly with over 50 cellular and viral proteins, some of which play roles, such as repair and recombination (Taylor and Knipe 2004). In addition, we have shown that UL12 interacts with Nbs1, a component of the MRN complex known to play an important role in cellular homologous recombination (Balasubramanian and Weller, manuscript in preparation). Taken together, the ability of ICP8 and UL12 to mediate strand annealing and strand transfer and to interact with viral and cellular repair and recombination proteins is consistent with the suggestion that DNA recombination plays a role in HSV DNA replication (reviewed in [Wilkinson and Weller 2003]). Further experimental evidence will be required to test this model.

HSV DNA Replication at the Cellular Level

HSV-1 DNA replication occurs in large globular replication compartments (RCs) in the nucleus of infected cells, and viral gene expression and DNA replication are thought to occur within these domains (Knipe 1989). ICP8 is believed to play a major role in nuclear events leading to the formation of RCs (Taylor and

Knipe 2003), and recent work by Everett and colleagues has led to a more refined model of the earliest steps of infection (Everett and Murray 2005; Everett et al. 2007, 2004). HSV genomes enter the nucleus and appear to cause the recruitment of cellular proteins of cellular ND10 proteins. ND10 (nuclear domains 10, also known as promyelocytic leukemia nuclear bodies or PODs) are defined by the accumulation of PML and many other cellular proteins involved in growth control, gene expression, and possibly DNA recombination and the DNA damage response (Dellaire and Bazett-Jones 2007, 2004). The genomes of several DNA viruses which replicate in the nucleus including SV40, adenoviruses, and other herpesviruses such as HCMV also appear to recruit ND10 proteins (reviewed in [Everett 2006]). The formation of ND10-like foci at viral genomes may reflect an antiviral cellular mechanism to repress expression of the viral genome, consistent with reports that many ND10 proteins are transcriptional repressors and are found at silenced regions of the chromatin (regions of heterochromatin) (Fernandez-Capetillo et al. 2003; Turner 2007; Turner et al. 2005). In support of this notion, PML was found to play a role in mediating the antiviral effects of IFN treatment in both HSV and HCMV infections (Everett 2006; Tavalai et al. 2006). Alternatively or in addition, it is possible that ND10 components are attracted to viral genomes because the genome is seen as a damaged DNA molecule in need of repair; many ND10 proteins have functions in DNA repair and recombination (Dellaire and Bazett-Jones 2007, 2004). Thus ND10 components may play dual roles in responding to damage and induction of silencing.

HSV Induces Disruption of ND10s

HSV is believed to counter the antiviral action of ND10 recruitment by disrupting ND10 by the action of ICP0, an immediate-early gene product. ICP0 is an E3 ubiquitin ligase (Boutell et al. 2003, 2002), and its ability to disrupt ND10 is thought to be a result of its ability to degrade sumoylated isoforms of PML and SP100, two components of ND10. The disruption of ND10 and the removal of at least some ND10 proteins may relieve the repressive activities of some ND10 proteins and thus provide an environment conducive to viral gene expression and viral DNA replication.

Prereplicative Sites

Replication compartments (RC) form rapidly during infection, and the only way to identify subassemblies of viral proteins important for RC formation is to freeze the progression of infection either by infection with viruses bearing mutations in replication proteins or in the presence of pharmacological agents which inhibit viral DNA synthesis (Burkham et al. 1998; Carrington-Lawrence and Weller 2003; Wilkinson and Weller 2004). We have defined five stages of infection based on the intracellular localization of viral and cellular proteins which are believed to play a

role in the formation of RCs. Stage I is defined by the recruitment of ND10 proteins to viral genomes as described above, and during this stage, no ICP8 foci can be detected by immunofluorescence microscopy. In cells that are in Stage II, ND10 have been disrupted and ICP8 can be detected. Although we originally reported that ICP8 is diffusely localized in Stage II, more sensitive microscopy has now revealed that microfoci of ICP8 are present at this stage and that these ICP8 microfoci are positioned adjacent to ICP4 foci (Livingston et al. 2008). Cells in Stage III contain a limited number of ICP8-containing foci whose formation is dependent on the presence of UL5, UL8, UL9, and UL52 (Burkham et al. 1998). Stage III can be divided into two: Stage IIIa foci contain the five viral proteins ICP8, UL5, UL8, UL9, and UL52, whereas Stage IIIb foci contain these five proteins along with HSV Pol, UL42, and PML. As mentioned above, the recruitment of the polymerase holoenzyme to the five protein scaffold requires the presence of an active primase subunit (Carrington-Lawrence and Weller 2003). If replication is allowed to proceed, replication compartments are observed which can be detected with both the ICP8 and the PML antibodies (stage IV). Thus, it appears that viral and cellular proteins assemble to prereplicative sites in an ordered manner to initiate viral DNA replication.

Host–Cell Interactions

Although the viral *cis*- and *trans*-acting factors necessary for viral replication have been identified, we know very little about the role of host proteins in viral DNA replication. Several cellular proteins are rearranged following viral infection: some are recruited into replication compartments and some are sequestered in foci adjacent to RCs called VICE (virus-induced chaperone-enriched) domains (Burch and Weller 2004; Wilkinson and Weller 2006). Several questions remain, however. Do the cellular proteins which are recruited to RCs such as RPA, Rad51, MRN proteins, and hsp90 play a direct role in viral DNA replication (Wilkinson and Weller 2006)? What is the function of the VICE domains, and why are some cellular proteins sequestered there, including the phosphorylated form of RPA, the ATR interaction protein (ATRIP), and the heat-shock protein hsc70? What are the roles of host proteins which have been identified as interaction partners for viral-replication proteins, such as the transcriptional coactivator HCF-1 (T. Kristie, personal communication), polymerase alpha-primase (Lee et al. 1995), and a neural F-box protein NFB42 which may play a role in ubiquitin-dependent degradation (Eom et al. 2004; Eom and Lehman 2003)? The biological significance of these interactions is not clear. It is possible that several cellular proteins play direct roles in either the initiation or the later stages of viral DNA replication. It is intriguing to speculate that the reason it has not been possible to recapitulate origin-dependent HSV DNA synthesis in vitro is that one or more host cellular proteins may be required. Cellular proteins which interact with viral proteins may be co-opted by the virus for various purposes such as subversion of antiviral defenses or the prevention of apoptosis. With the advent

of siRNA technology, it should be possible to address some of these unanswered questions about the involvement of host proteins in viral DNA replication.

Encapsidation of Viral Genomes

Viral DNA packaging into virions is a multistep process involving resolution of replication and/or recombination intermediates, specific cleavage events, packaging into preassembled capsids. The steps involved in this process are highly analogous to those of the more extensively studied DNA bacteriophages including (i) the formation of a procapsid intermediate consisting of a capsid shell initially supported by an internal scaffold, (ii) replacement of the internal scaffold with viral DNA, (iii) insertion of DNA through a unique portal vertex, and (iv) generation of unit length molecules by endonucleolytic cleavage of complex DNA concatemers by the activity of a two-component terminase. Several HSV gene products are involved in these steps including a terminase composed of the UL15 and UL28 proteins and a portal protein (UL6), which forms an oligomeric ring through which the viral DNA is taken up during the packaging reaction (reviewed in [Baines and Weller 2004]).

Summary

Although *cis*- and *trans*-acting viral proteins have been identified and their functions determined, many questions about the actual mechanism of HSV DNA replication and the involvement of host proteins remain unanswered. It is important to address these questions in part because viral proteins required for viral DNA replication provide very attractive targets for antiviral chemotherapy, and agents such as acyclovir and its derivatives which target the viral thymidine kinase and the viral DNA polymerase have been very successful. As with many other therapies, however, drug resistance is a very real threat which limits efficacy. Because most of the replication proteins discussed in this chapter are common to all the Herpesviridae, it is anticipated that new information generated here will be applicable to all herpesviruses. The helicase/primase has already been exploited as an antiviral target: two classes of highly potent helicase/primase inhibitors have been reported recently (Kleymann, 2003 #1608).

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