HERPES SIMPLEX VIRUS DNA REPLICATION

Paul E. Boehmer

Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey 07103

I. R. Lehman

Department of Biochemistry, Beckman Center, Stanford University School of Medicine, Stanford, California 94305-5307

ABSTRACT

The *Herpesviridae* comprise a large class of animal viruses of considerable public health importance. Of the *Herpesviridae*, replication of herpes simplex virus type-1 (HSV-1) has been the most extensively studied. The linear 152-kbp HSV-1 genome contains three origins of DNA replication and approximately 75 open-reading frames. Of these frames, seven encode proteins that are required for origin-specific DNA replication. These proteins include a processive heterodimeric DNA polymerase, a single-strand DNA-binding protein, a heterotrimeric primosome with 5′-3′ DNA helicase and primase activities, and an origin-binding protein with 3′-5′ DNA helicase activity. HSV-1 also encodes a set of enzymes involved in nucleotide metabolism that are not required for viral replication in cultured cells. These enzymes include a deoxyuridine triphosphatase, a ribonucleotide reductase, a thymidine kinase, an alkaline endo-exonuclease, and a uracil-DNA glycosylase. Host enzymes, notably DNA polymerase α-primase, DNA ligase I, and topoisomerase II, are probably also required.

Following circularization of the linear viral genome, DNA replication very likely proceeds in two phases: an initial phase of theta replication, initiated at one or more of the origins, followed by a rolling-circle mode of replication. The latter generates concatemers that are cleaved and packaged into infectious viral particles. The rolling-circle phase of HSV-1 DNA replication has been reconstituted in vitro by a complex containing several of the HSV-1 encoded DNA replication enzymes. Reconstitution of the theta phase has thus far eluded workers in the field and remains a challenge for the future.
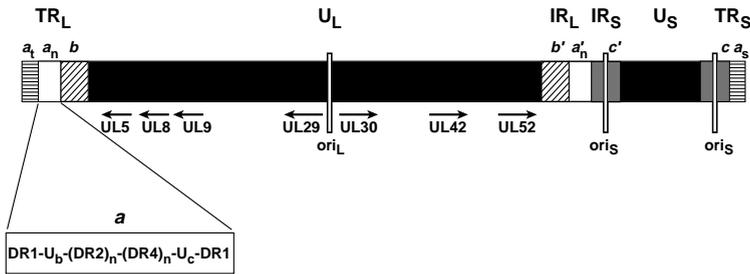
347

CONTENTS

INTRODUCTION

The *Herpesviridae* comprise a large family of viruses (more than 100 are known) that infect virtually all vertebrates including man. The human herpes viruses include herpes simplex virus type-1 (HSV-1), herpes simplex virus type-2 (HSV-2), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), human herpes virus 6 (HHV-6), human herpes virus 7 (HHV-7), and human herpes virus 8 (HHV-8). The human herpes viruses are significant pathogens and are therefore of substantial public health importance. HSV-2, for example, is the cause of a severe genital infection; VZV causes chicken pox in children and shingles upon reactivation from latency; and EBV is associated with infectious mononucleosis and Burkitt's lymphoma. Even those herpes viruses that produce rather benign infections in normal individuals can cause severe disease in immunocompromised patients, e.g. retinitis (HCMV) and Kaposi's sarcoma (HHV-8) in AIDS patients (reviewed in 1).

The *Herpesviridae* all have a similar structure consisting of a core containing a linear double-stranded DNA genome ranging in size from 120 to 230 kbp; an icosadeltahedral capsid, approximately 100 nm in diameter, containing 162 capsomers; an amorphous layer surrounding the capsid known as the tegument; and an envelope containing glycoprotein spikes protruding from its surface (reviewed in 2). The *Herpesviridae* promote lytic infection of susceptible cells,

TR_L U_L IR_L IR_S U_S TR_S

a_t a_n b b' a'_n c' c a_s

← UL5 ← UL8 ← UL9   ← UL29 $\downarrow$ UL30   UL42 →   UL52 →

ori_L ori_S ori_S

a

DR1-U_b-$(DR2)_n$-$(DR4)_n$-U_c-DR1

Figure 1 Structure of the herpes simplex virus type-1 genome. Diagrammatic representation of the HSV-1 genome. The positions of the *a*, *b*, and *c* repeats within the terminal repeats (TR_L and TR_S) and internal repeats (IR_L and IR_S), and the positions of the DNA replication origins (ori_L and ori_S) are as indicated. The position and direction of transcription of the seven essential DNA replication genes are indicated by the arrows. The enlarged area shows the composition of a unit-length *a* sequence, consisting of direct repeats (DR) 1, 2, and 3, and unique (U) domains b and c. Abbreviations: U, unique; $_t$, terminal; $_n$, variable number of copies; ′, inverted orientation; $_L$, long; $_S$, short. Not to scale, see text for details.

resulting in the production of infectious virus. They are also able to enter a latent state in which no infectious progeny are produced. Upon presentation of the appropriate stimulus, the latently infected cells enter a lytic phase with the resultant production of infectious virus particles. Of the *Herpesviridae*, HSV-1 has been the most extensively studied and is the focus of this review. Its 152-kbp genome is composed of two unique regions termed U_L (unique long) and U_S (unique short) that are flanked by repeated regions, *a*, *b*, and *c* (Figure 1; reviewed in 2).

The HSV-1 genome exists as an equimolar mixture of the four isomers that are generated by inversion of one or both unique regions via recombination. The *a* sequences appear to play an important role in the inversion because noninverting mutants of HSV-1 are generated by deletion of a segment of DNA that includes the internal *a* sequence (3). Such mutants can replicate in cell cultures, suggesting that inversion is not essential for viability (4). The HSV-1 genome contains not one but three origins of DNA replication: ori_L situated in the U_L segment of the genome, and two copies of ori_S in the two *c* sequences that flank the U_S segment (5, 6). The structure and function of these origins is considered below.

Upon penetration of the nucleus, a temporally regulated program of viral gene expression mediated by RNA polymerase II begins. A virion tegument protein, VP16, acts in *trans* together with the cellular octamer DNA-binding protein, oct-1, to induce the α or Immediate Early genes, the first set of HSV-1 genes to be expressed. There are five α gene products: ICP0, ICP4, ICP22,

ICP27, and ICP47, all of which function as transactivators of β or Early gene expression. Their synthesis peaks 2–4 hours postinfection. Functional α proteins are required for the synthesis of all the virally encoded proteins. Synthesis of the β class of proteins reaches peak rates 5–7 hours postinfection and is detectable as early as 3 hours postinfection. The β proteins include the enzymes that are required for replication of the viral genome: DNA polymerase; single-strand DNA-binding protein (SSB), also known as Infected Cell Polypeptide 8 (ICP8); DNA helicase-primase; origin-binding protein (UL9 protein); and those enzymes involved in nucleotide metabolism. Homologues of these proteins are found in virtually all herpes viruses.

Viral DNA synthesis begins shortly after the appearance of the β proteins and is detectable as early as 3 hours and continues up to 15 hours postinfection, with the bulk of DNA synthesized during the latter part of this period (7–10). The temporal program of viral gene expression ends with the appearance of the γ or Late proteins, which constitute the structural proteins of the virus (reviewed in 2).

HSV-1 DNA REPLICATION STRATEGY: A MODEL FOR THE REPLICATION OF THE HSV-1 GENOME

Soon after infection, the linear viral genome circularizes and DNA replication initiates at an origin. DNA replication initially proceeds by a theta mechanism and subsequently switches to a sigma or rolling-circle mode to yield long head-to-tail concatemers. Multiple DNA replication forks that arise from homologous recombination, *a* sequence–mediated genome isomerization, and other events, lead to the formation of an extensive network of branched DNA intermediates. Finally, these structures are resolved into unit-length genomes and packaged into preassembled capsids. What follows is a review of the evidence in support of this model.

Structure of Replicating DNA

As early as 0.5 hours postinfection and in the absence of protein synthesis, viral DNA accumulates in the nucleus and adopts an "endless" configuration whose structure is consistent with circularization (11–18). Although the mechanism of genome circularization has not been established, it may involve a recombination event. One clear advantage of this intermediate is that it eliminates the need for a specialized mechanism to replicate the termini. It was recently shown that HSV-1 replication is inhibited at the nonpermissive temperature in a cell line with a thermolabile regulator of chromosome condensation, RCC1 (19). In these cells, the viral genome fails to circularize, suggesting that RCC1 is involved in genome circularization and that circularization is a prerequisite

for DNA replication. The circularized genome presumably acts as a template for origin-dependent theta replication. To date, there is no direct evidence of unit-length circular DNA or theta replication intermediates. However, there are several lines of evidence that support a sigma or rolling-circle mechanism of replication accompanied by the formation of complex branched intermediates later in infection.

The prevalence of endless DNA until the very late stages of replication was the primary reason for suggesting a rolling-circle mechanism (11–16). Replicating HSV-1 DNA consists of rapidly sedimenting species with *S* values greater than that of mature DNA (13). Restriction enzyme digestion indicates that these species consist of head-to-tail concatemers (18, 20). Direct examination of HSV-1 DNA replication intermediates by electron microscopy revealed molecules that contain extensive regions of single-stranded DNA, DNA replication forks, loops, and branched DNA structures (21–23). More recently, HSV-1 DNA replication intermediates have been examined by pulsed-field gel electrophoresis (18). This analysis showed that replicating DNA consists of highly branched DNA networks. The existence of these branched DNA replication intermediates was confirmed by two-dimensional gel electrophoresis and is consistent with the DNA networks observed by electron microscopy (24). These branches consist of Y- and X-junctions that likely represent DNA replication forks and recombination intermediates or merging replication forks, respectively. Further evidence for a rolling-circle mechanism is provided by the presence of large head-to-tail concatemers of plasmids that contain an HSV-1 origin in cells coinfected with HSV-1 (5). Furthermore, experiments in vitro show that fractionated nuclear extracts that contain a complex of the HSV-1 DNA replication proteins promote leading- and lagging-strand rolling-circle replication of model DNA templates (25–27).

While the existence of these replication intermediates is indicative of a rolling-circle mechanism that leads to the formation of highly branched DNA structures, there is no information on how DNA replication switches from a presumed theta mode to a rolling-circle mode or on what *trans-* and *cis-*acting factors are required for this transition.

Cleavage and Packaging of DNA

Cleavage and packaging of concatemeric DNA replication intermediates into preformed capsids is a tightly coupled process in which DNA cleavage occurs once a capsid is filled with one genome equivalent. It involves *cis-*acting elements within the viral *a* sequence, the action of several viral gene products, and possibly some host cell factor(s).

Analysis of viral DNA ends indicates that the cleavage reaction introduces an asymmetric cut in the *a* sequence, producing an L terminus that contains 18 bp

of the DR1 repeat and a single 3′-nucleotide extension, and an S terminus that contains 1 bp of DR1 and a single 3′-nucleotide extension. Circularization of these ends reconstitutes a complete DR1 sequence that is shared between the terminal a sequences of the L and S components (28). The elements within the a sequence that are responsible for cleavage and packaging are located in the U_b and U_c domains and are designated pac1 and pac2, respectively (29).

It has been suggested (29) that, during cleavage and packaging, concatemeric viral DNA is bound at the pac sites by a complex of proteins that includes a component of the capsid. The cleavage-packaging apparatus then spools the DNA into the capsid while it "scans" the DNA for the next a sequence of like polarity. Once two direct copies of the a sequence are juxtaposed, a recombination event produces linear genomic DNA by deleting the DNA between the direct repeats of the a sequence while an amplification event restores the continuity of the DNA concatemer for subsequent cycles of cleavage and packaging.

Although the molecular mechanism of the cleavage-packaging reaction is unknown, several biochemical activities that may be involved in this process have been identified. These include virus-encoded proteins that specifically recognize the pac2 site (30), and a virus-induced DNA endonuclease that introduces double-strand cuts in the U_c domain of the a sequence (31, 32) and is part of an activity that promotes in vitro recombination of repeated a sequences (33). In addition, mutational studies have implicated several nonstructural HSV-1 gene products (UL6, UL15, UL25, UL28, UL32, and UL33 proteins) as well as several capsid proteins (UL18, UL19, and UL26.5 proteins) (34–40). Moreover, the alkaline endo-exonuclease encoded by the *UL12* gene may be involved in processing branched DNA replication intermediates into packagable linear concatemers (41–43).

Origins of DNA Replication

The HSV-1 genome contains three *cis*-acting elements that function as origins of DNA replication: one copy of ori_L, located between the diverging *UL29* and *UL30* genes within U_L, and two copies of ori_S, located within the c sequence of the internal repeat short (IRS) and the terminal repeat short (TRS), between the diverging *ICP4* and *ICP22/47* genes (5, 6).

Some of the earliest evidence for the existence of DNA replication origins in the genome of HSV-1 was based on the observation of DNA replication bubbles within U_L and TRS/IRS that coincide with the positions of the origins (22). Further evidence came from experiments in which newly synthesized DNA was mapped to the U_L-U_S junction, which is the site of ori_S (23).

Subsequent evidence for the existence of distinct origins came from the analysis of defective viral genomes that are generated upon repeated passage of the virus at high multiplicities of infection. These defective genomes possess

DNA sequence elements that allow amplification and packaging. Defective genomes were classed into two groups, designated Class I and II, on the basis of the origin of their DNA sequences (44–52). Both Class I and II molecules contain DNA sequences derived from TRS. Class I molecules also contain DNA sequences from U_S, while Class II molecules contain additional sequences derived from the center of U_L. Molecules of either class could be replicated upon coinfection with wild-type HSV-1, supporting the presence of DNA replication origins in these defective genomes (49, 52, 53).

Recombinant plasmids containing DNA sequences from Class I defective genomes were examined for autonomous replicating sequence (ARS) activity in cells that were coinfected with HSV-1. This led to the identification of an ∼100-bp region within the *c* sequence of IRS and TRS that possessed ARS activity, designated ori_S (5, 54, 55). The origin contained within the Class II defective genomes (52) was mapped to a 425-bp region between the *UL29* and *UL30* genes and shown to possess ARS activity in cells coinfected with HSV-1 and designated ori_L (6). In transient assays, the ARS activity of both ori_S and ori_L is stimulated by flanking transcription factor binding sites that are part of the diverging *ICP4* and *ICP22/47* (ori_S) or *UL29* and *UL30* (ori_L) genes (56, 57).

The pertinent features of ori_S and ori_L are illustrated in Figure 2. Both ori_S and ori_L contain large palindromes of 45 and 144 bp, respectively, that center around an A + T region of 18 and 20 bp. Flanking the A + T region are inverted repeats that are binding sites for the HSV-1 encoded origin-binding protein (UL9 protein). On each side of the A + T region of ori_L is a high affinity UL9 protein binding site, designated Box I. In contrast, ori_S possesses one copy of Box I, 5′ to the A + T region, and a homologous sequence with a 10-fold lower affinity for UL9 protein, designated Box II, 3′ to the A + T region. In ori_L, the Box I sites are flanked by homologous sequences, designated Box III, that differ from Box I by only one residue but act as low affinity recognition sites for the UL9 protein. In ori_S there is only one copy of Box III, 5′ to Box I. The interaction of the UL9 protein with these sites is discussed below. Since the large
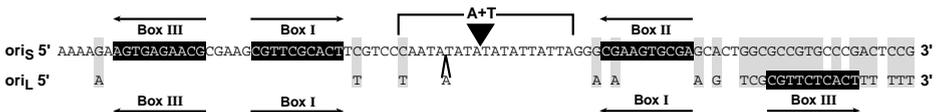
Figure 2 Structure of the herpes simplex virus type-1 origins of DNA replication, ori_S and ori_L. The DNA sequence of the minimal ori_S is shown. Boxed bases indicate sequence differences between ori_S and ori_L. The arrows depict the relative orientation of the UL9 protein recognition sites, Boxes I, II, and III. The triangle indicates the center of symmetry. A + T, A + T sequence. See text for details.

palindrome within ori_L is prone to deletion upon propagation in *Escherichia coli* (6, 57), most of our understanding of the functional organization of the HSV-1 origins is based on ori_S. Studies of ori_S have shown that mutations in Box I that abolish UL9 protein-binding in vitro eliminate origin activity in vivo (58–60). Similar mutations in Box II greatly reduce origin activity (58–60). Although, as noted above, Box III differs from the high affinity Box I by only one residue, UL9 protein-binding to isolated Box III is undetectable. Nevertheless, Box III does play a role in origin activity because mutations in Box III reduce replication to approximately 20% of wild-type levels (58–60). In summary, efficient replication from ori_S requires that the two high affinity UL9 protein binding sites, Boxes I and II, be intact. Models of how DNA replication initiates at ori_S are discussed below.

It is unclear why the HSV-1 genome contains multiple origins that appear identical in every respect other than size and location. The existence of two copies of ori_S may be rationalized by its location in a repeated region (IRS/TRS) of the genome. However, neither copy of ori_S is essential, since deletion of one or both copies of ori_S has no effect on viral DNA replication in cultured cells (10, 61, 62). It has been hypothesized that one type of origin is activated during theta replication, while the other is required to initiate sigma DNA replication. However, there are no distinguishing features of ori_S and ori_L that would suggest differential utilization of the origins. In a search for cellular proteins that may influence origin activity, it was shown that both minimal ori_S and ori_L are targets for a nuclear protein, designated OF-1, whose recognition sequence is homologous to that of the CCAAT family of DNA-binding proteins (57, 63, 64). The significance of the OF-1 binding sites in ori_S and ori_L remains unclear. In transient assays, both origins replicate with similar kinetics and to similar extents (57). It is also possible that the two classes of origins are active at different phases of the viral life cycle, one during lytic infection and the other during reactivation from latency. However, deletion of ori_L has no effect on reactivation from latency (62). Thus, the HSV-1 origins appear redundant in function but are nevertheless conserved for an as yet unknown reason.

It has been found that a 1.6-kbp DNA sequence that maps to the *UL39* gene possesses ARS activity in the absence of virus encoded functions (65). It is possible therefore that this DNA sequence functions as an origin that is replicated by cellular factors during latency in neurons. However, this prediction remains to be proven.

In conclusion, while there is no proof of the existence of theta replication intermediates that initiate at one of the HSV-1 origins, it has been shown that genome circularization is a prerequisite for viral replication and that replication requires either ori_S or ori_L and the action of a viral protein that possesses all the properties of an initiator protein (UL9 protein). These observations strongly

suggest that HSV-1 DNA replication initiates by a theta mechanism and make it unlikely that other mechanisms, such as inter- or intramolecular recombination or transcription, all of which could potentially occur on linear DNA templates, are responsible for the initiation of HSV-1 DNA replication.

HSV-1 GENE PRODUCTS ESSENTIAL FOR ORIGIN-SPECIFIC DNA REPLICATION

The use of temperature-sensitive mutants in the identification of essential HSV-1 genes revealed several complementation groups with defects in DNA synthesis (66–75). Some temperature-sensitive mutations were mapped to the genes for the DNA polymerase (68, 76–82) and SSB (ICP8) (73, 74, 82–88). The development of a transient DNA replication assay in which origin-containing plasmids are replicated by transfected HSV-1 sequences that supply *trans*-acting factors led to major advances in the identification of the essential HSV-1 DNA replication genes (89). This analysis permitted identification of seven HSV-1 genes that are necessary and sufficient for origin-specific DNA replication: *UL5, UL8, UL9, UL29, UL30, UL42,* and *UL52* (90, 91). These results were subsequently confirmed by demonstrating that mutations in the *UL5* (92–94), *UL8* (95), *UL9* (96, 97), *UL42* (98), and *UL52* (99) genes abrogate viral replication. Although the functions of the *UL29* (ICP8) and *UL30* (DNA polymerase) gene products had already been identified, the remaining five genes encoded proteins of unknown function. These genes were subsequently correlated with proteins identified by biochemical analyses. The functions of these gene products are summarized in Table 1.

Infection of insect (Sf9) cells with recombinant baculoviruses that express the seven essential DNA replication genes can support the replication of origin-containing plasmids (100). This observation supports earlier findings and suggests that any host functions required for this process are sufficiently conserved to allow DNA replication in insect cells.

The following is a detailed review of the biochemical properties of the HSV-1 DNA replication proteins and of a model of their presumed function in DNA replication.

Origin-Binding Protein (UL9 Protein, OBP)

A search for proteins that bind to an HSV-1 origin led to the identification of an HSV-1-induced factor that recognized sequences within ori_S (101). Further purification of this sequence-specific DNA-binding activity led to the identification of an 83-kDa protein that bound to two inverted repeats that flank the A + T region within ori_S, designated Boxes I and II (Figure 2) (102). Subsequently, it was demonstrated that the origin-binding protein was the product of

Table 1 HSV-1 DNA replication proteins

Essential DNA replication proteins Protein	Gene	Size (kDa)	Activities
DNA polymerase	*UL30*	136	DNA polymerase, 3′-5′ exonuclease, RNase H
	UL42	51	Double-strand DNA-binding protein, DNA polymerase processivity factor
Single-strand DNA binding protein ICP8	*UL29*	128	Single-strand DNA-binding protein Stimulates DNA polymerase, helicase-primase, and UL9 protein
DNA helicase-primase	*UL5/52*	99/114	5′-3′ DNA helicase, DNA primase
	UL8	80	Stimulates primer synthesis, interacts with ICP8 and UL9 protein
Origin-binding protein	*UL9*	94	Origin-binding protein, 3′-5′ DNA helicase

Nonessential DNA replication proteins Protein	Gene	Size (kDa)	Activities
Alkaline endo-exonuclease	*UL12*	68	Endo- and exonuclease
Uracil N-glycosylase	*UL2*	36	Uracil-DNA glycosylase
Deoxyuridine triphosphatase	*UL50*	39	Deoxyuridine triphosphatase
Thymidine kinase	*UL23*	41	Nonspecific nucleoside kinase
Ribonucleotide reductase	*UL39/40*	140/38	Protein kinase, ribonucleotide, reductase

the *UL9* gene (103). The *UL9* gene encodes an 851–amino acid polypeptide (UL9 protein) with a calculated mass of 94,246 Da (104). Amino acid sequence analysis of the UL9 protein led to the identification of conserved ATP-binding and DNA helicase motifs (105, 106) that have been shown to be essential for viral replication (97, 107, 108). The UL9 protein exists as a homodimer both in solution and when bound to its target sequences (109–114). The dimerization of UL9 protein is mediated through the N-terminal part of the protein (115, 116), presumably through a leucine-zipper motif encompassing residues 150–171 (117). The sequence-specific DNA-binding activity of the UL9 protein resides in the C-terminal 317 amino acids (117–120). Unlike the full-length UL9 protein, this 37-kDa C-terminal DNA-binding domain exists as a monomer in solution (115). Expression of this DNA-binding domain has a dominant negative effect on viral replication, presumably owing to its ability to occupy the origin nonproductively (100, 108, 122). An open-reading frame that localizes and overlaps with the *UL9* gene, designated *UL8.5* (which is expressed with delayed-early kinetics, in contrast to the *UL9* gene, which is an Early gene product) encodes a 53-kDa nuclear protein, designated OBPC, that contains the

C-terminal DNA-binding domain of the UL9 protein (123, 124). It is possible that this protein plays a role in the regulation of viral replication by preventing initiation of origin-specific DNA replication at later times postinfection.

Further analysis of the interaction of UL9 protein with ori_S showed that in addition to binding to Boxes I and II (102, 103, 125), binding to a third homologous site (Box III) could be detected (115, 126). Examination of the binding of UL9 protein to ori_S by electron microscopy revealed the presence of large nucleoprotein complexes that were frequently involved in intermolecular interactions in which two DNA molecules were joined by one protein complex (127). The binding of UL9 protein to ori_S appears to be enhanced by nucleoside triphosphates (109, 128). The precise DNA recognition site for the UL9 protein in Box I was mapped to a 10-bp sequence (5′ CGTTCGCACT) (125, 126, 129, 130). Homologous sequences, in inverted orientations, constitute the binding sites of Boxes II and III (Figure 2). The UL9 protein is predicted to interact with the bases of the major grove (130), and since Boxes I and II are separated by three helical turns of the DNA duplex, UL9 protein dimers are probably arranged in inverted orientations on the same side of the DNA helix. Finally, the observation that Boxes I and II are each occupied by UL9 protein dimers (114) suggests that the recognition sites are bipartite, consisting either of overlapping inverted repeats or asymmetric recognition sites. It should be noted, however, that studies of the interaction of a histidine-tagged C-terminal portion of the UL9 protein with Box I showed a 1:1 stoichiometry of binding (131).

The UL9 protein binds to isolated Box I with apparent dissociation (k_d) and association (k_a) constants of 0.1 and 0.3 nM, respectively (102, 130). Divergence from the Box I recognition sequence in Boxes II and III decreases the binding affinity of the UL9 protein for these sites. Thus, UL9 protein has an ∼10-fold lower affinity for Box II (102, 130), while binding to Box III occurs with even lower affinity and no apparent sequence specificity (126). However, as noted above, in the context of the entire ori_S, specific binding to Box III is detectable (115, 126). Furthermore, mutations within the UL9 protein recognition sequence that greatly reduce binding to isolated Boxes I or II have little effect on the ability of UL9 protein to interact with these sites in the context of the entire ori_S (126, 128). This cooperativity is attributed to protein-protein interactions between ori_S-bound UL9 protein dimers. The inability of the C-terminal DNA-binding domain of UL9 protein to stabilize binding to mutant sites within ori_S suggests that this cooperativity is mediated through the N-terminus of the UL9 protein (115). Interruption of the leucine-zipper within the N-terminal domain of the UL9 protein disrupts cooperative binding to ori_S (116). Nevertheless, the observation that the monomeric C-terminal DNA-binding domain of UL9 protein binds cooperatively to its recognition sites as a dimer suggests that certain protein-protein interactions are mediated through the C-terminal domain (114).

In addition to its sequence-specific DNA-binding activity, the UL9 protein possesses DNA-stimulated nucleoside triphosphatase and DNA helicase activities (111, 112, 132, 133). The ability of UL9 protein to hydrolyze ATP and dATP, and to a lesser extent CTP, dCTP, and UTP (111, 112), correlates with their ability to support DNA unwinding (112, 133). The K_m for ATP in the ATPase reaction of the UL9 protein is $0.54 \pm 0.15\ \mu M$, while the K_m for DNA is $1.12 \pm 0.27\ \mu M$ and $10.3 \pm 6.0\ \mu M$ for single- and double-stranded DNA, respectively (134). The ATPase activity of the UL9 protein is strongly influenced by the length and nature of the DNA cofactor, favoring long polymers with minimal secondary structure (111, 112, 132). The minimum length required to elicit ATPase activity is 14 nucleotides (132), which correlates with a minimum length of 15 nucleotides required for sequence-specific DNA binding (130) and may thus represent the DNA-binding-site size. Both enzymatic activities are optimally active at pH 8.5–9.5, $\sim$2.5 mM $MgCl_2$, 45°C and can be inhibited by increasing ionic strength to greater than 50 mM NaCl (132, 133). DNA unwinding proceeds with a 3′-5′ directionality (112, 133) and depends on the integrity of the DNA strand along which the UL9 protein translocates, but not on that of the opposing strand (135). DNA unwinding requires a single-stranded DNA loading site; unwinding of blunt-ended DNA substrates has not been detected. The unwinding of DNA by the UL9 protein appears to be stoichiometric rather than catalytic, involving the assembly of an active helicase complex (133). The helicase activity is greatly and specifically stimulated by the HSV-1 SSB, ICP8 (112, 133). In the absence of ICP8, DNA unwinding is limited to $\sim$200 bp (133). The addition of ICP8 increases the rate and extent of DNA unwinding, yielding products up to 3 kb (110, 133).

The ability of the UL9 protein to recognize elements within the origin of replication and to act as a DNA helicase suggests that it functions as a DNA replication initiator protein whose role is to make the DNA at the origin accessible to the DNA replication machinery. In these respects, UL9 protein resembles the large tumor antigens (Tags) of simian virus 40 (SV40) and polyoma virus, and the E1 proteins of bovine and human papilloma virus (reviewed in 136–138). Accordingly, as expected of an initiator protein, the UL9 protein has been shown to induce structural changes in the DNA at the origin. Binding of the UL9 protein to ori_S induces hypersensitivity to DNase I in the A + T region (126). In addition, using a replication-competent HSV-2 mutant ori_S that contains a 23-bp A/T insertion in the A + T sequence, it was shown that the UL9 protein induces hypersensitivity to DNase I and micrococcal nuclease in the A + T region (139). This hypersensitivity occurred with a periodicity of $\sim$10 bp that is attributed to looping of the DNA. Hypersensitivity to DNase I in the A + T region requires protein-protein interactions between UL9 protein dimers bound to Boxes I and II (113). Furthermore, in a reaction that depends on the free energy of supercoiled DNA, it was shown that UL9 protein can induce sensitivity to

potassium permanganate in the A + T region (139). On the basis of these observations, it was proposed that protein-protein interactions between ori_S-bound UL9 protein dimers lead to looping and distortion of the A + T region.

Further examination of the interaction of UL9 protein with ori_S by electron microscopy has led to an understanding of the mechanism by which the origin is activated to allow subsequent DNA replication (109). This analysis showed that, consistent with previous results, the UL9 protein bound to ori_S as a pair of dimers and bent the DNA by ∼86°. In the presence of ATP, stem-loop structures of up to ∼1 kb in size were extruded from the base of the UL9 protein complex. Furthermore, the DNA in the stem loops could be stabilized by photo-cross-linking and was bound by *E. coli* SSB with high affinity (109). These observations led to the following model of origin-specific DNA unwinding (109; Figure 3): Protein-protein interactions between UL9 protein dimers bound to Boxes I and II lead to bending, looping, and distortion of the A + T region. These interactions prevent ATP-dependent translocation of the UL9 protein dimers, causing the DNA to be pulled through the protein complex and spooled out as single-stranded DNA. Interactions between the UL9 protein and ICP8 presumably stimulate this reaction and stabilize unwound regions of DNA. Likewise, interactions between UL9 protein and the UL8 subunit of the HSV-1 DNA helicase-primase or the 180-kDa subunit of DNA polymerase α-primase could lead to recruitment of a primase to initiate DNA synthesis (see below).

It has recently been observed that the UL9 protein, together with ICP8, can unwind specifically Box I of ori_S if an appropriately oriented 3′ single-stranded tail of at least 18 nucleotides (the binding site size for ICP8) is attached to Box I (SSK Lee & IR Lehman, unpublished observations). This finding suggests that a complex consisting of the UL9 protein and ICP8 bound to single-stranded DNA constitutes the specific helicase that can separate the two strands, permitting entry of the DNA replication machinery. The single-stranded DNA binding site for ICP8 in ori_S may be provided by the A + T sequence that links Boxes I and II. Because of its relatively weak hydrogen bonding, this sequence may be easily unwound, possibly as a consequence of the initiation of transcription at the promoters that flank the origins. It is worth noting that this mode of unwinding of an HSV-1 origin by the UL9-protein-ICP8 single-stranded DNA complex apparently differs from that seen with the SV40 Tag, in which the Tag itself is the origin-specific helicase and the SSB plays a purely passive role, binding the single strands generated by helicase action and thereby preventing their reannealing.

Single-Strand DNA-Binding Protein, ICP8

ICP8 was one of the first HSV-1 DNA replication proteins to be identified. It was subsequently shown to bind preferentially to single-stranded DNA (83). ICP8 is the product of the *UL29* gene (82) and consists of 1196 amino acids
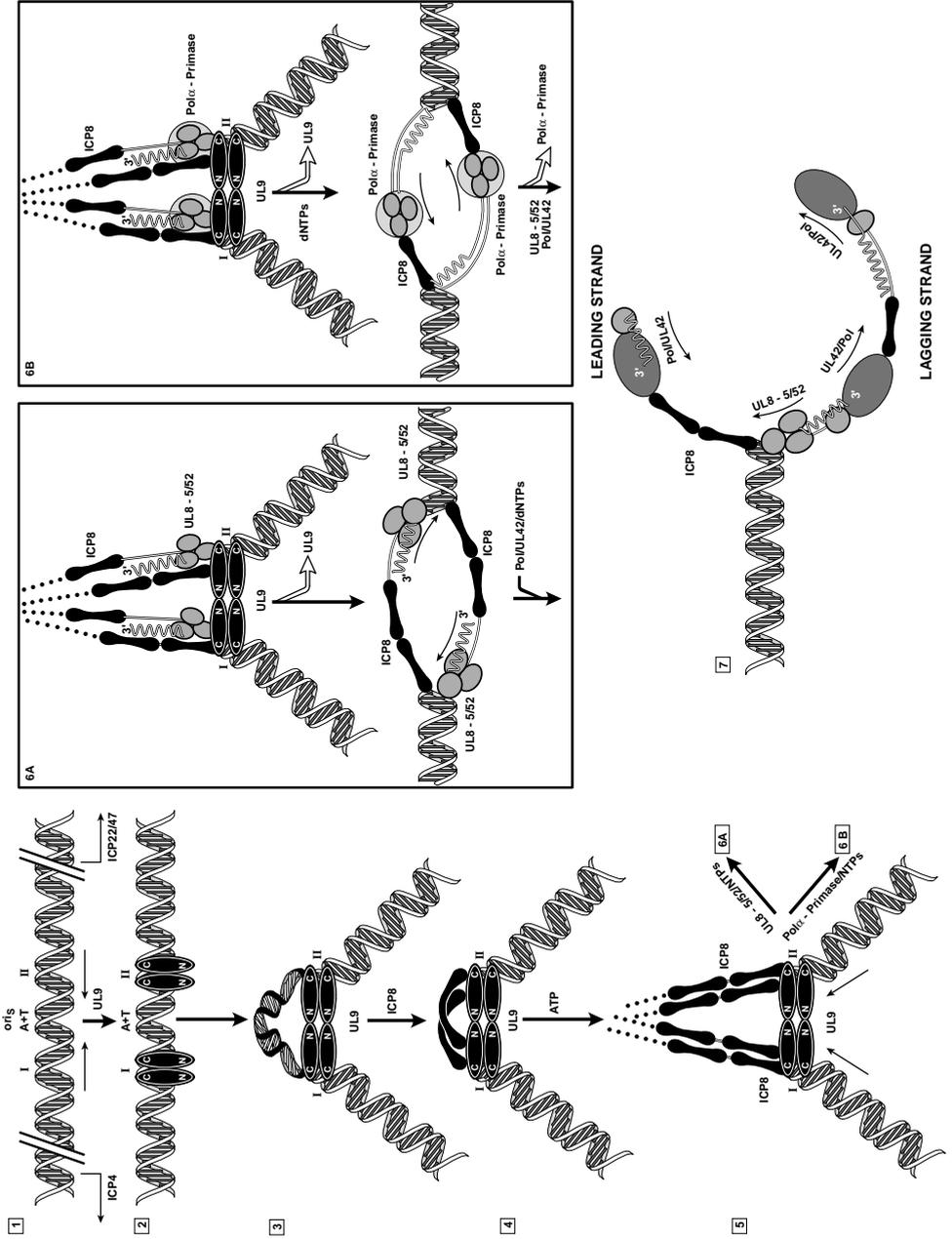
with a calculated molecular mass of 128,342 Da. Amino acid sequence analysis of ICP8 reveals a region, encompassing residues 803–849, that contains a consensus DNA-binding sequence, and a Cys-X_{2-5}-Cys-X_{2-15}-Cys/His-X_{2-4}-Cys/His–type zinc-finger motif (140) from residues 499–512 (141, 142). Mutagenesis of Cys-499 and -502 produces a nonfunctional protein that fails to complement a temperature-sensitive *UL29* mutant at the nonpermissive temperature (141). Subsequent studies have shown that ICP8 contains equimolar amounts of tightly chelated zinc (143). Zinc is not required for DNA binding but rather for the structural integrity of ICP8. Further biochemical analysis has shown that DNA binding involves contacts with free sulfhydryl groups and surface lysine and tyrosine residues (144, 145). Fluorescence quenching at 336 nm in the presence of single-stranded DNA suggests that tryptophan residues are also involved in DNA binding (145). Genetic and biochemical techniques have been used to map the DNA-binding domain of ICP8 to a region encompassing residues 300–849, which contains both the zinc-finger motif and the consensus DNA-binding sequence (141, 142, 146).

ICP8 binds single-stranded DNA rapidly and cooperatively and with at least fivefold greater affinity than double-stranded DNA (147, 148). The ability of poly(A) to compete for binding suggests that ICP8 can also interact with certain RNAs (149). Optimal single-stranded DNA binding occurs at pH 7.6 (149) and 150 mM NaCl (148). Examination of ICP8 binding to single-stranded DNA, by electron microscopy and negative staining, showed regular protein-DNA filaments in which the DNA is held in an extended configuration (110, 149). Estimates of the DNA binding site size for ICP8 based on the ratio of ICP8 required for stimulation of the HSV-1 DNA polymerase, strand-displacement and -annealing activities, and for nuclease protection, all fall in the range of

←——————————————————————————————————

Figure 3   Model of herpes simplex virus type-1 DNA replication. *1–3*: successive binding, looping, and distortion of ori$_S$ by the UL9 protein (UL9). I, II, and A + T represent the UL9 protein recognition sites, Boxes I and II, and the A + T region, respectively. The converging arrows indicate the relative orientation of Boxes I and II. The diverging arrows indicate transcription from the ICP4 and ICP22/47 promoters. *4*: binding of ICP8 to the UL9 protein and distorted DNA. *5*: ATP-dependent DNA unwinding that generates ICP8-coated DNA strands. *6A*: recruitment of DNA helicase-primase (UL8-5/52) by UL9 protein followed by primer synthesis (*curved line*) and dissociation of UL9 protein. *6B*: recruitment of DNA polymerase α-primase (Pol α-Primase) by UL9 protein followed by primer synthesis (*curved line*) and elongation, and dissociation of UL9 protein and DNA polymerase α-primase. *7*: Unwinding of the DNA replication fork and lagging-strand priming by the DNA helicase-primase. Leading- and lagging-strand DNA synthesis promoted by the HSV-1 DNA polymerase (Pol/UL42). The arrows indicate the direction of translocation of the DNA replication proteins or that of the DNA. Abbreviations: NTPs, nucleoside triphosphates; dNTPs, deoxynucleotide triphosphates. See text for details.

one ICP8 to 12–22 nucleotides (150–153). These values are in agreement with a site size of 15–18 nucleotides estimated by electron microscopy (110).

Like the *E. coli* SSB and the bacteriophage T4 gene 32 protein, ICP8 can reduce the melting temperature of synthetic polynucleotides such as poly(dA)-poly(dT) (87, 154). The helix-destabilizing activity of ICP8 is manifested by its ability to unwind short regions of duplex DNA in an ATP- and direction-independent manner (150). This activity is inhibited by increasing $MgCl_2$ and ionic strength, which presumably act to stabilize the DNA duplex. The reaction is extremely rapid and cooperative and requires saturating concentrations of ICP8. In this respect ICP8 resembles the eukaryotic SSB [replication protein A (RP-A)], which also possesses strand-displacement activity (155, 156).

ICP8, like other SSBs, can also catalyze the renaturation of complementary single strands (151). Reannealing requires $MgCl_2$ and is optimal at 75 mM NaCl and pH 7.6. Duplex DNA formation increases with ICP8 concentration, reaching a maximum at saturating concentrations, and is second order with respect to DNA concentration.

The ability of ICP8 to strand-displace and reanneal complementary single strands must in part be responsible for the strand-transfer activity of ICP8 (157) and may account for the high frequency of homologous recombination observed in HSV-1–infected cells. The strand-transfer activity of ICP8 is characterized by its ability to transfer a DNA strand from a linear duplex to a complementary single-stranded DNA circle in a reaction that requires $MgCl_2$ but not (d)NTPs (157).

ICP8 also appears to be involved in the regulation of viral gene expression, functioning both as a negative and positive regulator of γ or Late gene expression (158–161). Finally, ICP8 participates in multiple protein-protein interactions that affect the activities of the DNA polymerase, DNA helicase-primase, and UL9 protein. Moreover, ICP8 plays a key role in the assembly of the HSV-1 DNA replication proteins into prereplicative sites that are precursors to discrete nuclear locations in which viral DNA replication occurs. These aspects of ICP8 are discussed in the section on Protein-Protein Interactions.

DNA Helicase-Primase

The search for additional HSV-1–specific DNA replication enzymes led to the identification of a virus-induced DNA helicase (162). This enzyme was purified from the nuclei of HSV-1–infected cells as a virus-specific DNA-dependent ATPase that was further distinguished from host DNA-dependent ATPases by its ability to also hydrolyze GTP. This enzyme was shown to unwind short oligodeoxyribonucleotides annealed to single-stranded M13 DNA with a 5′-3′ directionality, suggesting that it translocates along the lagging strand, unwinding the DNA at the replication fork (162). The directionality of the

helicase was later confirmed using partially duplex oligodeoxyribonucleotides with a 5′-terminal single-stranded tail (MS Dodson, personal communication). Further purification of the helicase showed that it consists of three subunits, with molecular masses of 120, 97, and 70 kDa, that are the products of the *UL52, UL5,* and *UL8* genes, respectively (163). In addition, it was shown that this multisubunit enzyme possesses primase activity (163).

The *UL5*, *UL8*, and *UL52* genes encode proteins of 882, 750, and 1058 amino acids with molecular masses of 98,710; 79,921; and 114,416 Da, respectively (91). Amino acid sequence analysis of the UL5 protein led to the identification of conserved ATP-binding and DNA helicase motifs (105, 106) that have been shown to be essential for viral replication (93, 94). The interaction of the UL5 subunit with ATP was confirmed by demonstrating that purified UL5 protein alone exhibits low levels of DNA-dependent ATPase activity (164). Moreover, mutagenesis of Gly-815-Ala within helicase motif V of UL5 protein reduces the DNA-dependent ATPase activity of the mutant holoenzyme in vitro (165). Residues 610–636 of the UL52 protein contain a proposed divalent metal-binding motif that is conserved in DNA polymerases and primases (166, 167). Site-directed mutagenesis of this motif inactivates the primase activity of the mutant holoenzyme in vitro and abolishes origin-specific DNA replication in vivo, indicating that it comprises the primase active site (166, 167).

The holoenzyme consists of a 1:1:1 association of the *UL5*, *UL8*, and *UL52* gene products, with a native molecular mass of ∼270 kDa (168, 169). A sub-assembly that consists of the UL5 and UL52 subunits retains DNA-dependent ATPase, helicase, and primase activities and therefore constitutes the core enzyme (170, 171). In contrast, the UL8 protein lacks detectable enzymatic or DNA-binding activities (171, 172).

A steady state kinetic analysis of the helicase-primase holo- and core enzymes revealed the presence of two distinct nucleoside triphosphatase sites. The high affinity site hydrolyzes exclusively ATP with a K_m of 170 μM, while the other site hydrolyzes both ATP and GTP with a K_m in the mM range (173). In addition, these sites are activated by different concentrations of DNA cofactor. NTP hydrolysis at either site can support DNA helicase action, although maximal activity is observed at high (mM) concentrations of ATP. Further support for the existence of multiple nucleoside triphosphatase sites is provided by the observation that mutagenesis of Gly-815-Ala within helicase motif V of the UL5 protein causes a threefold reduction in k_{cat} and K_m for DNA without affecting the DNA helicase or primase activities (165). However, it is still unclear how these sites contribute to the mechanism of the enzyme.

A detailed analysis of the helicase activity of the helicase-primase has yet to be performed. To date our knowledge is based on the unwinding of short oligodeoxyribonucleotides annealed to single-stranded M13, although the

holoenzyme has been shown to unwind a 2.3-kbp nicked plasmid in an ICP8-dependent manner (169). The helicase is optimally active in the range of pH 7.0–7.5 and 1.5 mM $MgCl_2$ (174), and it is inhibited at ionic strengths greater than 100 mM NaCl (G Villani & PE Boehmer, unpublished data).

The primase is optimally active at pH 8.5, and 2.5 mM $MgCl_2$ (169). The products of primase action are oligoribonucleotides that are 6–13 bases in length (164, 169, 175). Using single-stranded ϕX174 as template, it was subsequently shown that the primase exhibits sequence specificity. The preferred template sequence was mapped to 3′ AG*C*CCCTCCCA, directing the synthesis of a 10-mer oligoribonucleotide, starting at the italicized C residue (176).

The UL8 protein was shown to stimulate primer synthesis by the UL5/52 core enzyme (174–176). Thus, while UL8 protein has no effect on the K_m for DNA (15 μM nucleotides) of the UL5/52 core enzyme, it increases the rate of primer synthesis approximately threefold (175). In contrast, there is no detectable effect of the UL8 protein on the rate of the DNA-dependent ATPase and helicase activities of the core enzyme (170, 171, 174). However, it has been shown that the UL8 subunit is required for optimal DNA helicase, DNA-dependent nucleoside triphosphatase, and primase activity in the presence of ICP8 (174; M Gustafsson & IR Lehman, unpublished results).

DNA Polymerase

The induction of a virus-specific DNA polymerase in HSV-1–infected cells was first demonstrated in the mid 1960s (177, 178). Since that time, the HSV-1 DNA polymerase has been intensely studied, both as a model eukaryotic DNA polymerase and as a target for antiviral drugs. The DNA polymerase was initially identified as a 144–150-kDa protein (179, 180). It was subsequently shown that the DNA polymerase is a 1235–amino acid, 136,413-Da protein that is encoded by the *UL30* gene (82, 85, 181, 182). The UL30 protein exhibits significant sequence similarity to other viral and cellular DNA polymerases including human DNA polymerase α-primase, *Saccharomyces cerevisiae* DNA polymerase δ, *E. coli* DNA polymerase I, and bacteriophage T4 DNA polymerase (183–185).

HSV-1 DNA polymerase purified from virus-infected cells exists as a heterodimer in which the UL30 protein is associated with an ∼65-kDa protein (186–188). The ∼65-kDa protein was subsequently identified as the product of the *UL42* gene, encoding a 488–amino acid, 51,154-Da phosphoprotein that possesses double-strand DNA-binding activity (91, 189). The interaction between the UL30 and UL42 proteins occurs with an association constant of 1×10^8 M^{-1} (190) and has been shown to be essential for viral replication (121, 191–193). The essential nature of this interaction has led to growing interest in its potential as a novel antiviral drug target, using rationally designed

compounds that can mimic the protein interface and thus prevent heterodimerization (194, 195).

Early characterization of the heterodimeric DNA polymerase showed that deoxynucleotide polymerization is optimal between pH 8.0 and 8.5 (177); is stimulated by salt, with optimal activity at $\sim$0.1 M NaCl (180, 196, 197); and is resistant to 0.15 M $(NH_4)_2SO_4$ (198), which is inhibitory to cellular DNA polymerases. DNA polymerase activity is also stimulated by polyamines (196, 199, 200). A steady state kinetic analysis of deoxynucleotide incorporation showed the K_m for deoxynucleoside triphosphates (dNTPs) to be approximately 0.5 μM (196, 201).

The HSV-1 DNA polymerase possesses 3′-5′-exonuclease activity that is intrinsic to the UL30 subunit (179, 196–198, 200, 202–205). The 3′-5′-exonuclease is active under conditions that are optimal for deoxynucleotide polymerization and uses a variety of DNA substrates including gapped DNA, DNA hairpin structures, and single-stranded oligodeoxyribonucleotides (197, 200). In the absence of dNTPs, the 3′-5′-exonuclease is twice as active at removing 3′-unpaired deoxynucleotides as 3′-paired deoxynucleotides (197). In contrast, in the presence of all four dNTPs, there is no detectable 3′-5′-exonuclease on paired 3′-deoxynucleotides, presumably due to continuous polymerization, whereas 3′-unpaired deoxynucleotides are completely removed (197). Consequently, the 3′-5′-exonuclease serves as a proofreading activity to ensure high fidelity of DNA replication.

The UL30 protein contains three sequence motifs that are homologous to and align with exonuclease sequence motifs I, II, and III of *E. coli* DNA polymerase I and other DNA polymerases (184, 185, 206). Mutagenesis of Asp-368-Ala in the putative 3′-5′-exonuclease sequence motif I of the UL30 protein drastically reduces its 3′-5′-exonuclease activity (185, 205). Furthermore, the inability to construct a recombinant virus bearing this mutation suggests that the 3′-5′-exonuclease of the HSV-1 DNA polymerase is essential for viral replication (185). Interestingly, mutagenesis of exonuclease motif II (184) produces an enzyme that lacks detectable DNA polymerase activity, suggesting that this region is also involved in deoxynucleotide polymerization (207).

The HSV-1 DNA polymerase holoenzyme has also been shown to function as a ribonuclease that can degrade the RNA strand of RNA:DNA hybrid substrates (188). Using an in situ assay, it was demonstrated that this RNase H activity is associated with the 136-kDa UL30 subunit (188). This observation was confirmed by demonstrating that recombinant 136-kDa UL30 protein as well as an $\sim$80-kDa N-terminal proteolytic fragment of the UL30 protein also possess RNase H activity (152, 203, 204). The RNase H activity was initially attributed to a 5′-3′-exonuclease associated with the HSV-1 DNA polymerase (188). However, it now appears that the potent 3′-5′-exonuclease of the UL30

protein may be responsible for this activity because no 5′-3′-exonuclease activity was detectable in a wild-type or recombinant UL30 protein that lacks 3′-5′-exonuclease activity (205). It is also possible that preparations of HSV-1 DNA polymerase holoenzyme exhibiting 5′-3′-exonuclease activity were contaminated with the HSV-1 alkaline endo-exonuclease (UL12 protein) that exhibits a potent 5′-3′-exonuclease (179, 208). This possibility is supported by the finding that immune serum directed against the UL12 protein can inhibit the 5′-3′-exonuclease activity observed in preparations of HSV-1 DNA polymerase, and by the ability to separate chromatographically these two activities (179, 208).

Efforts to delineate the regions of the UL30 protein that are required for DNA polymerase and 3′-5′-exonuclease activities showed that deletion of residues 881–959 abolished DNA polymerase activity in vitro and failed to complement in vivo (209). Indeed, amino acid substitutions (Gly-885-Arg, Asp-886-Asn, Thr-887-Lys, Asp-888-Ala, and Gly-896-Val) in conserved motif 3 (184) of the UL30 protein abolish DNA polymerase activity (209). In contrast, deletion of residues 1073–1144, 1177–1235, 1–27, 1–67, or 1–227 has no effect on DNA polymerase activity (209, 210). Consistent with these observations, immunological studies have shown that antibodies raised against the central and C-terminal portions of the HSV-1 DNA polymerase inhibit both DNA polymerase and 3′-5′-exonuclease activities, while antibodies to residues 1072–1146 had little effect (211, 212).

The UL42 protein acts to increase the processivity of the UL30 DNA polymerase (152, 213, 214). It is a functional homologue of the DNA polymerase clamps, which include the β subunit of E. coli DNA polymerase III (215, 216), the eukaryotic proliferating cell nuclear antigen (PCNA) (reviewed in 217), and bacteriophage T4 gene 45 protein (reviewed in 218). These proteins form a ring that encircles the DNA duplex, and interact with their cognate DNA polymerase to prevent dissociation of the enzyme from the primer-template, thereby ensuring high processivity of deoxynucleotide polymerization. However, these DNA polymerase clamps lack DNA-binding activity, and their assembly on the DNA is mediated by another set of proteins, the clamp loaders (reviewed in 217). In contrast, the HSV-1 DNA polymerase is tethered to the DNA by the double-strand DNA binding activity of the UL42 protein itself. This model is supported by the finding that stimulation of DNA polymerase activity requires the DNA-binding activity of the UL42 protein (219).

Studies of the interaction of the UL42 protein with a primer-template have shown that the UL42 protein increases the affinity of UL30 protein for a 3′-terminal primer approximately 10-fold (220). DNase I footprinting experiments with the UL30 protein showed that it binds to the primer-template junction, covering 14 bp of duplex DNA and 18 bases of upstream single-stranded DNA. The addition of UL42 protein extends the footprint in the duplex portion of the

DNA substrate, supporting the view that the UL42 protein acts as a clamp that reduces the dissociation of UL30 protein from the DNA (220). This conclusion is further supported by a pre–steady state kinetic analysis of single deoxynucleotide incorporation with a defined primer-template by the UL30 protein and DNA polymerase holoenzyme. The UL42 protein was found to reduce the k_d for the enzyme-DNA complex 30-fold (3.2 ± 1.4 s^{-1} for the holoenzyme as compared with 0.1 ± 0.01 s^{-1} for the UL30 protein). Surprisingly, the UL42 protein also increased the k_a fivefold. Neither dNTP binding nor the chemical step was affected (BD Song & D Herschlag, personal communication).

The site of interaction between the UL30 and UL42 proteins was initially mapped to the C-terminal 227 residues of the UL30 protein. Deletion of this region had no effect on DNA polymerase activity but abolished UL42 protein binding and stimulation of DNA polymerase activity (183). Further examination showed that the residues required for physical association and functional stimulation by UL42 protein localize to the extreme C-terminus of UL30 protein, encompassing residues 1209–1235 (108, 191, 192, 194, 195). The site of interaction in the UL42 protein is less defined. Overlapping peptides that span the entire UL42 protein are largely nonspecific in their ability to disrupt the functional interaction between the UL30 and UL42 proteins (221, 221a). However, it was subsequently shown that the N-terminal 340 residues of the UL42 protein are sufficient to bind duplex DNA, interact with UL30 protein, and support viral replication (191, 192, 222, 223). In addition, it was shown that DNA-binding activity, physical association with UL30 protein, and functional stimulation of DNA polymerase reside in different regions of the UL42 protein. Thus, DNA binding localizes to the C-terminus of the 340-residue region, while stimulation of DNA polymerase resides in two regions, residues 129–163 and residues 202–337, the latter being required for physical association with UL30 protein (191, 192, 222). The involvement of multiple regions of UL42 protein is further supported by the identification of two protease-resistant peptides, derived from N-terminal regions of UL42 protein, that are required for the functional and physical interaction with UL30 protein (190).

Numerous nucleotide analogues [9-(2-hydroxyethoxymethyl)guanosine 5′-triphosphate (acyclovir triphosphate), 9-β-D-arabinofuranosyladenosine 5′-triphosphate (araATP), aphidicolin, (E)-5-(2-bromovinyl)-2′-deoxyuridine 5′-triphosphate], and pyrophosphate analogues (phosphonoacetate, phosphonoformate, oxalate) have been examined for their ability to inhibit viral replication in vivo and to inhibit selectively the HSV-1 DNA polymerase in vitro. This has led to the development of several antiviral drugs, notably acyclovir, that are of therapeutic value and that were also instrumental in the initial mapping of the HSV-1 DNA polymerase gene (181). Thus, mutants that exhibit resistance or altered sensitivity to nucleotide analogs—aphidicolin (80, 224–227), acyclovir

(81, 228–232), araATP (79, 81)—have been shown to map to the *UL30* gene, as do those that exhibit resistance to phosphonoacetate (76, 77, 80, 81, 85, 231, 233–235).

The mapping of amino-acid changes in the UL30 protein of drug-resistant mutants has also provided some information on the localization of the dNTP and pyrophosphate binding sites. Thus, drug resistance localizes to conserved DNA polymerase motifs (184) 1, 2a, and 2b (203, 225, 235–239) and to several nonconserved regions encompassing residues 577–637 (237), Val-258 (225), and Asp-531 (227). These data indicate that while the conserved motifs are clearly involved in substrate recognition, distant sites in the primary sequence probably interact to contribute to the binding site.

Finally, drug resistance in the *UL30* gene has been observed to lead to an antimutator phenotype (240), in which increased DNA replication fidelity is due not to an increase in $3'-5'$-exonuclease activity, but rather to an increase in selection specificity of dNTPs prior to incorporation (241).

PROTEIN-PROTEIN INTERACTIONS

An emerging theme in DNA replication is the importance of specific protein-protein interactions. These interactions serve to assemble multiprotein complexes that promote efficient DNA replication. There have been numerous reports of both functional and physical interactions among the HSV-1 DNA replication proteins. We assume that they are manifestations of a multiprotein complex or replisome that mediates replication of the genome. A plausible conjecture is that the assembly of this complex is initiated by the ability of the UL9 protein to bind to the high affinity recognition site, Box I, within the origins. Protein-protein interactions between UL9 protein dimers are subsequently responsible for cooperative binding to the lower affinity recognition sites. Thereafter, origin-bound UL9 protein may recruit other DNA replication proteins via specific protein-protein interactions. In the first instance, UL9 protein probably recruits ICP8, which has been shown to greatly stimulate the DNA helicase and DNA-dependent ATPase activities of the UL9 protein in a species-specific manner (112, 132, 133; SSK Lee & IR Lehman, unpublished data). This functional interaction is a manifestation of a physical association that is mediated through the C-terminal DNA-binding domain of UL9 protein (242). Although ICP8 and the C-terminal DNA-binding domain of the UL9 protein associate to form a heterodimer in the absence of DNA, the stoichiometry of DNA-bound ICP8-UL9 protein complex is unclear (131). Deletion of the 27 C-terminal residues of UL9 protein greatly reduces ICP8 binding and results in loss of stimulation of DNA unwinding by ICP8 (243). In addition, this mutation reduces origin-specific DNA replication in vivo approximately 100-fold

(243). These observations suggest that the interaction between ICP8 and UL9 protein is important for origin-specific replication. We hypothesize that ICP8 is recruited to origin-bound UL9 protein where it stimulates DNA unwinding and contributes to the destabilization of the DNA duplex and the maintenance of the single-stranded conformation (150; SSK Lee & IR Lehman, unpublished results). Once the DNA at the origin has been unwound, the ICP8-UL9 protein complex dissociates as a result of the change in conformation of the DNA. This finding is supported by the existence of an ICP8-UL9 protein complex in the presence of duplex DNA but not single-stranded DNA (131).

The interaction between the UL9 protein and ICP8 during DNA unwinding has been examined by electron microscopy (110). These studies have revealed a novel mechanism of DNA unwinding that occurs in several discrete steps. First, UL9 protein localizes to the boundary of single- and double-stranded DNA. Second, UL9 protein translocates internally along the DNA duplex in an ATP-dependent manner, resulting in localized DNA unwinding and rewinding. Third, UL9 protein recruits ICP8 to localized regions of unwound DNA, causing rapid and cooperative unwinding of the entire DNA fragment, resulting in intertwined protein-coated strands of DNA. Fourth, polymerization of ICP8 along the DNA causes dissociation of UL9 protein and separation of complementary single strands, resulting in the formation of ICP8-coated single strands. This mechanism of DNA unwinding is distinct from models in which DNA unwinding initiates at a DNA end and proceeds by progressive movement of a helicase at the fork.

Following unwinding of the origin, the next plausible step in the replication pathway is the recruitment of a primase. In this regard, it has been shown that the UL9 protein can interact with the UL8 subunit of the HSV-1 DNA helicase-primase (244). This interaction is mediated through the N-terminal part of the UL9 protein and may serve to recruit a primase activity to the origin. However, it has been shown that the minimal ori_S does not act as a template for the primase activity of the DNA helicase-primase (176). Consequently, consistent with the ability of the UL9 protein to cause extensive DNA unwinding at the origin (109), sequences outside the immediate origin may act as templates. Alternatively, a different primase may be involved in initiating DNA synthesis. This notion is supported by the finding that the UL9 protein interacts with the 180-kDa subunit of human DNA polymerase α-primase (245). Consequently, it is possible that DNA polymerase α-primase is responsible for synthesizing the initiating primer and for its elongation. However, it has been shown that aphidicolin-resistant HSV-1 can replicate in cells at concentrations of aphidicolin that inhibit deoxynucleotide polymerization by DNA polymerase α-primase in vitro (225, 226, 246). In addition, strains of HSV-1 resistant to the nucleoside analog 9-β-D-arabinofuranosyladenosine (araA) have been isolated

(79, 81). The triphosphate derivative of this drug is an effective inhibitor of the DNA polymerase and primase activities of DNA polymerase α-primase (247). These findings suggest that HSV-1 can replicate in the presence of drugs that inhibit DNA polymerase α-primase. However, the inhibition of DNA polymerase α-primase by aphidicolin and araATP under these conditions may not be complete, and residual activity could still account for the participation of DNA polymerase α-primase in HSV-1 DNA replication.

We suggest that after origin-specific DNA unwinding and synthesis of the initiating primer, the UL9 protein dissociates from the DNA, and unwound regions of DNA are coated with ICP8. Additional DNA replication enzymes are then recruited to the DNA by ICP8. ICP8 can stimulate the HSV-1 DNA polymerase (149, 154), and ICP8 is required for processive DNA synthesis by the DNA polymerase holoenzyme (152). These observations may not reflect solely the ability of ICP8 to remove secondary structure in DNA templates that may impede the progress of the DNA polymerase, but rather may be manifestations of a physical interaction between ICP8 and the DNA polymerase (186, 248, 249). Further evidence for an interaction between ICP8 and the DNA polymerase is provided by genetic studies that show that mutations in the *UL29* gene alter the drug sensitivity of the DNA polymerase (250).

In the replication of the *E. coli* or SV40 chromosomes, either an asymmetric dimeric DNA polymerase (*E. coli*) or two different DNA polymerases, DNA polymerase α-primase and DNA polymerase δ (SV40), are needed to promote the leading- and lagging-strand DNA synthesis required for semidiscontinuous DNA replication (251). At present there is no evidence for an asymmetric, dimeric HSV-1 DNA polymerase. However, a dimer consisting of the highly processive UL30-UL42 protein complex to catalyze leading-strand synthesis, and the UL30 protein alone with its relatively low processivity to catalyze lagging-strand synthesis, may serve to promote semidiscontinuous HSV-1 DNA replication. In this model, the DNA helicase-primase may act in concert with the DNA polymerase lacking the UL42 protein. Another possibility is that one of the cellular DNA polymerases—DNA polymerase α-primase, DNA polymerase δ, or DNA polymerase ε—may act together with the HSV-1 DNA polymerase.

While the UL9 protein is presumably involved in recruiting a primase to initiate DNA synthesis, it appears that interactions between ICP8 and the DNA helicase-primase operate at the DNA replication fork. Thus, ICP8 has been shown to stimulate the DNA helicase activity of the DNA helicase-primase in a species-specific manner (169, 174; M Gustafsson & IR Lehman, unpublished data) in a process that requires the UL8 subunit (174). Similarly, the utilization of ICP8-coated DNA templates for primase action requires the UL8 subunit (174). These observations are indicative of an interaction between ICP8 and

the UL8 subunit of the DNA helicase-primase that ensures concomitant DNA unwinding and priming at the replication fork.

Consistent with the requirement for a topoisomerase in relieving torsional stress ahead of the DNA replication fork, it was reported that topoisomerase activity copurifies with the HSV-1 DNA polymerase (252). However, upon reexamination, no evidence was found for the induction of either topoisomerase I or II in HSV-1–infected cells (253, 254). On the other hand, it was recently shown that viral replication is greatly reduced in cells treated with a specific topoisomerase II inhibitor (255). These results are consistent with a role for topoisomerase II in the decatenation of theta replication intermediates.

Interactions among the alkaline endo-exonuclease, ICP8, and DNA polymerase (208, 249, 256) support the notion that the alkaline endo-exonuclease may function in the processing of branched DNA replication intermediates. Indeed, it has been shown that the alkaline endo-exonuclease specifically interacts with ICP8 and that they colocalize to sites of DNA replication in the nuclei of infected cells (249, 257, 258).

Further evidence for the existence of multiple protein-protein interactions during HSV-1 DNA replication is provided by cytological examination of HSV-1–infected cells by immunofluorescence. Soon after infection, and prior to the onset of DNA replication, the HSV-1 DNA replication proteins assume a scattered distribution in the nucleus, presumably dictated by the presence of nuclear localization signals contained within each protein or multisubunit complex (259–265). Approximately 2–3 hours postinfection, ICP8 localizes to numerous punctate nuclear sites known as prereplicative sites (266). Similar observations were made when DNA replication was blocked by using either replication-deficient mutants or DNA replication inhibitors (259, 262, 266). Likewise, in the absence of viral DNA replication, UL30 protein, DNA helicase-primase, and UL9 protein also localize to prereplicative sites (262, 267, 268). It has been hypothesized that the transition from a diffuse nuclear distribution to prereplicative sites correlates with the assembly of a multiprotein complex that is poised to initiate DNA replication. It was demonstrated that localization of UL30 protein to prereplicative sites depends on a functional ICP8 (262). More recently, it was shown that specific protein-protein interactions between ICP8, UL9 protein, and the subunits of the DNA helicase-primase are required for the assembly of these proteins into prereplicative sites and that recruitment of the DNA polymerase into this complex is mediated by the UL42 subunit (267, 268). These data suggest that interactions among UL9 protein, presumably bound to an origin; ICP8; and the DNA helicase-primase result in the formation of a complex that initiates origin-specific DNA unwinding and primer synthesis. Subsequent DNA synthesis is initiated by recruiting the HSV-1 DNA polymerase by an interaction involving the UL42 subunit.

Upon the onset of DNA replication, approximately 3–4 hours postinfection, the distribution of the HSV-1 DNA replication proteins changes from prereplicative sites to globular nuclear domains known as replication compartments that are the sites of viral DNA replication (259, 261, 262, 267–269). Several cellular DNA replication proteins including DNA polymerase α-primase, DNA ligase I, RP-A, and PCNA, as well as p53 and retinoblastoma protein have been shown to colocalize with ICP8 and may therefore play a role in viral replication (270). Finally, a partially purified complex consisting of the DNA helicase-primase, ICP8, and DNA polymerase holoenzyme has been shown to promote rolling-circle DNA replication of plasmid molecules (25–27).

Collectively these data support the existence of a multiprotein complex that functions in the replication of the HSV-1 genome. Based on these data, we have devised several models on how DNA replication initiates at an HSV-1 origin and how the DNA replication fork is established (Figure 3).

HSV-1 GENE PRODUCTS NOT ESSENTIAL FOR ORIGIN-SPECIFIC DNA REPLICATION

In addition to ICP8, DNA polymerase, UL9 protein, and DNA helicase-primase, all of which are essential for HSV-1 DNA replication, the HSV-1 genome encodes a set of enzymes whose function is not required for its replication in cultured cells. They undoubtedly serve some function in the viral life cycle. These include an alkaline endo-exonuclease, a uracil-DNA glycosylase, a deoxyuridine triphosphatase, a thymidine kinase, and a ribonucleotide reductase.

Alkaline Endo-Exonuclease

The alkaline endo-exonuclease was one of the first of the virally encoded enzymes to be purified (177). The nuclease is an abundant 67,503-Da phosphoprotein with an apparent mass of 85 kDa encoded by the *UL12* gene (104). As its name implies, it exhibits both endo- and exonuclease activities and is optimally active at pH 9–10. It shows no sequence specificity (271).

Although DNA replication is unimpaired in a null mutant in the *UL12* gene, and Late viral proteins are expressed, there is a defect in the production of infectious virus, suggesting that the nuclease may play a role in the processing or packaging of viral DNA into infectious virions. More specifically, the defect appears to be in the formation of DNA-containing capsids; these form in the nucleus of the HSV-1–infected cells and mature into the cytoplasm (41).

Uracil N-Glycosylase

The deamination of cytosine in DNA to form uracil is a mutagenic event resulting in the conversion of a G-C to an A-T base pair. Repair of this lesion is

promoted by uracil-DNA glycosylase, an enzyme that catalyzes the cleavage of the N-glycosylic bond linking the uracil to deoxyribose in the DNA backbone. The resulting abasic or AP site is then repaired by the combined action of AP endonuclease, DNA polymerase, and DNA ligase (272). HSV-1 encodes its own uracil-DNA glycosylase, despite the presence of this enzyme in all mammalian cells (273). The HSV-1 uracil-DNA glycosylase is a 36,326-Da protein encoded by the *UL2* gene (104, 274). However, a truncated 27-kDa form of the enzyme expressed in *E. coli* using an internal start codon is fully active (275). The HSV-1 enzyme is 39% identical to the human and 49% identical to the *E. coli* enzyme (276). Its crystal structure, which has been solved to 1.75 Å resolution, has provided some insight into the basis for the specific cleavage of the N-glycosylic bond linking uracil to the deoxyribose sugar (277). The extreme specificity results from a combination of favorable pseudo–Watson-Crick interactions with uracil and the exclusion of normal DNA bases, in particular thymine. The crystal structure has also revealed a new fold, distantly related to that present in dinucleotide-binding proteins. Like the cellular uracil-DNA glycosylases, the viral enzyme is inhibited by 6-(p-alkylanilino) uracils; however, one of the uracil analogues, 6-(p-n-octylanilino) uracil, is highly selective for the viral enzyme.

Although, as noted above, the HSV-1 encoded uracil-DNA glycosylase is dispensable for viral replication in cultured cells, it appears to play some role during viral replication in the intact animal. Intracranial inoculation of uracil-DNA glycosylase–defective *UL2* mutants in mice produced a 10-fold reduction in neurovirulence compared with the wild-type virus. However, peripheral inoculation of the *UL2* mutant showed a 10^5-fold reduction compared with the wild-type virus. Examination of replication kinetics showed a reduced rate of viral replication in both the mouse peripheral and central nervous systems. Latency is established normally with the mutant virus. However, following transient hyperthermia, which induces the lytic state, the frequency of reactivation of the mutants was decreased. Restoration of the wild-type locus resulted in full neurovirulence and the ability to reactivate (278). These findings suggest that the HSV-1 uracil-DNA glycosylase plays a role during acute viral replication and in the reactivation from latency.

Deoxyuridine Triphosphatase

Deoxyuridine triphosphatase (dUTPase) catalyzes the specific hydrolysis of dUTP to dUMP and inorganic pyrophosphate. The enzyme thus provides a mechanism to prevent the incorporation of uracil into DNA and generates dUMP, the precursor of dTTP (251). The HSV-1 dUTPase, encoded by the *UL50* gene, has a molecular mass of 39,125 Da; it appears to be a monomer, in contrast to the cellular enzyme, which is a homodimer of 22.5-kDa subunits

(104, 279–281). dUTPase-deficient mutants of HSV-1 replicate with wild-type kinetics and achieve wild-type titers in cultured cells (282). However, like the uracil-DNA glycosylase mutants, they are 10-fold less neurovirulent than the wild-type following intracranial inoculation of mice and considerably less virulent following footpad inoculation. The dUTPase mutants replicate normally in the footpad and enter and replicate efficiently in the peripheral nervous system. However, their replication in the central nervous system is reduced. Although the mutant dUTPase strains can establish latency, they display greatly reduced reactivation. Neurovirulence, neuroinvasiveness, and reactivation are all restored by recombination with wild-type dUTPase sequences, suggesting that dUTPase does play a role in the HSV-1 infection cycle (283).

Thymidine Kinase

The HSV-1 thymidine kinase is more appropriately termed deoxypyrimidine nucleoside kinase to reflect its ability to catalyze the phosphorylation of deoxynucleosides in addition to thymidine (284). In fact, the HSV-1 thymidine kinase can phosphorylate various purine nucleosides, including a wide variety of ribo and deoxynucleoside analogues, and it even has thymidylate kinase activity (285). The multifunctional enzyme, which is a product of the *UL23* gene, is a homodimer consisting of two 40,918-Da polypeptides. The crystal structure of the enzyme complexed with thymidine and with gancyclovir has been solved (286). Its broad substrate specificity is the basis for the therapeutic action of the acyclic guanosine analogues, acyclovir and gancyclovir. Acyclovir is phosphorylated by the HSV-1 thymidine kinase to generate acyclovir diphosphate which, when further phosphorylated by cellular kinases, becomes a substrate for the HSV-1 DNA polymerase. Incorporation of acyclovir triphosphate results in chain-termination, and inactivation of the DNA polymerase, with the consequent inhibition of viral replication (287). The host thymidine kinase, because of its restricted substrate specificity, is unable to phosphorylate acyclovir in uninfected cells lacking the viral thymidine kinase (251).

As for the uracil DNA glycosylase and dUTPase, the HSV-1 thymidine kinase is not essential for viral replication in cell culture or in the intact animal. Upon corneal inoculation of mice with *UL23* deletion mutants, the mutants replicated to high titers in the eye. However, such mutants are defective in their replication in trigeminal ganglia. They do establish latency, but fail to reactivate, indicating that neither thymidine kinase activity nor ganglionic replication is necessary for the establishment of latency. It does, however, appear to be essential for reactivation from latency (288).

Ribonucleotide Reductase

The HSV-1 encoded ribonucleotide reductase consists of two nonidentical subunits of 124,043 and 38,017 Da encoded by the *UL39* and *UL40* genes,

respectively (104), that form an α_2-β_2 tetramer (289, 290). Like the cellular enzyme, the smaller subunit contains a tyrosyl-free radical that is essential for activity (291, 292). The large subunit has an intrinsic serine/threonine protein kinase activity that is capable of autophosphorylation but is incapable of phosphorylating exogenous proteins such as histones or calmodulin (293, 294). The function of the kinase activity is not known. HSV-1 does not encode thioredoxin, the hydrogen donor for ribonucleotide reduction, but uses the cellular protein instead (295). It therefore appears that the HSV-1 ribonucleotide reductase catalyzes ribonucleotide reduction by a mechanism very similar to the host cell enzyme. However, unlike the cellular enzyme, the viral ribonucleotide reductase is exempt from many of the allosteric controls that inhibit or activate the host enzyme, which permits it to respond to deoxynucleotide pools (296).

Interaction of the two subunits is dependent upon the seven C-terminal amino acids of the small subunit (297). This interaction has been examined in great detail with the aim of developing inhibitors of enzymatic activity that prevent association of the two subunits, thereby inhibiting viral replication. As an example, the modified pentapeptide Val-Val-Asn-Asp-Leu shows an IC_{50} of 180 nM, which approaches the range of therapeutic efficacy (298).

The HSV-1 ribonucleotide reductase is not essential for HSV-1 DNA or viral replication in rapidly dividing cultured cells (299). However, there is some evidence that it may be required for growth in nondividing cells such as neurons (300).

HOST ENZYMES IMPLICATED IN HSV-1 DNA REPLICATION

Although the HSV-1 encoded uracil-DNA glycosylase, dUTPase, and ribonucleotide reductase have their counterparts in the host and are presumably specifically required at some stage in the viral life cycle, the HSV-1 genome does not encode a DNA ligase or a DNA topoisomerase and must therefore utilize these cellular enzymes for its replication. As noted above, a cellular topoisomerase II inhibitor can prevent HSV-1 replication in vivo. It is possible that HSV-1 also requires the cellular DNA polymerase α-primase for the initiation of DNA replication.

RECONSTITUTION OF DNA REPLICATION IN VITRO

As described previously, studies of HSV-1 DNA replication in vivo have demonstrated that the linear 152-kbp genome circularizes shortly after infection of susceptible host cells, then enters a rolling-circle mode of DNA replication generating concatemeric DNA which is then cleaved and packaged as unit-length molecules. A bipartite mode of DNA replication of this kind has been observed for bacteriophage λ (302). Although, as noted above, rolling-circle

replication of the HSV-1 genome as well as of plasmids into which an HSV-1 origin has been inserted has been observed in vivo, there is as yet no compelling evidence in vivo for a theta mode of replication.

Numerous attempts have been made to observe origin (ori$_S$ or ori$_L$)-dependent DNA replication in vitro. None has thus far been successful. These attempts initially involved incubation of plasmids into which an HSV-1 origin has been inserted, with extracts of HSV-1 infected cells taken at various times postinfection under conditions (ATP and an ATP regeneration system; ribo- and deoxynucleoside triphosphates) that promote vigorous replication of comparable plasmids containing an SV40 origin. Subsequently, with the finding that the products of the *UL5, UL8, UL52, UL29, UL30, UL42,* and *UL9* genes are sufficient to promote the replication of origin-containing plasmids in vivo, the purified products of these genes were tested for their ability to promote DNA replication of ori$_S$ or ori$_L$-containing plasmids, and again no origin-dependent DNA replication was observed.

In contrast to the inability to reconstitute the theta mode of HSV-1 DNA replication, rolling-circle replication promoted by extracts of HSV-1–infected cells has been achieved. The first demonstration of rolling-circle replication made use of an artificial replication fork consisting of M13 single-stranded DNA to which a complementary oligonucleotide with an unpaired 5′ single-stranded tail had been annealed. Rolling-circle replication was observed both with crude extracts of HSV-1–infected cells and with a partially purified fraction containing the HSV-1 DNA polymerase and the UL5 and UL52 subunits of the DNA helicase-primase. ICP8 stimulated the reaction but was not essential (25). An estimate of the rate of fork movement of 250 nucleotides per minute was substantially below the in vivo rate of approximately 3000 for pseudorabies virus, another herpes virus (301), suggesting that additional factors are required.

In a second approach, a high-molecular-weight complex ($M_r > 10^6$) was isolated by gel filtration and ion-exchange chromatography from extracts of insect (Sf9) cells that had been infected with baculoviruses recombinant for the seven HSV-1 genes that are required for its replication. This complex, which consisted of the HSV-1 DNA polymerase, ICP8, and DNA helicase-primase, could promote the rolling-circle replication of circular plasmid templates to generate concatemeric DNA molecules up to four times unit-length as judged by gel electrophoresis and electron microscopy. Neither the UL9 protein nor an HSV-1 origin was required, and in fact, the presence of ori$_S$ within the plasmid inhibited DNA replication in the presence, but not the absence, of the UL9 protein (26).

Whole-cell extracts of HSV-1–infected human cells (293 cells) can also promote the rolling-circle replication of plasmid templates, in a reaction that closely resembles that observed with the high-molecular-weight complex isolated from

the baculovirus-infected Sf9 cells (27). Preliminary evidence suggests that a similar enzyme complex is present in the HSV-1–infected 293 cells. Although the rate of fork movement in these reactions has not been accurately determined, it appears to approach the in vivo rate cited above (R Skaliter & IR Lehman, unpublished data). However, the efficiency of the reaction is very low (1–2% of the template molecules replicated) for both the complex obtained from the baculovirus-infected Sf9 cells and the extract of HSV-1–infected 293 cells. The form of the plasmid DNA that serves as the template for rolling-circle replication is not known, and the small proportion of the template molecules replicated may reflect the low level of the appropriate template that is generated by either the enzyme complex or the crude extract. A single nick that could serve as a site for the initiation of rolling-circle replication does not appear to be involved because pretreatment of supercoiled plasmid did not increase the efficiency of the reaction. Possibly a recombinational event analogous to that which appears to be involved in the switch from theta to rolling-circle replication in bacteriophage λ may be required (302).

RELATIONSHIP BETWEEN RECOMBINATION AND DNA REPLICATION

Replication of the HSV-1 genome appears to be closely associated with homologous recombination. Stages at which recombination is clearly involved are in the *a* sequence–mediated inversion of the U_L and U_S segments, in the circularization of the viral genome following entry into the nucleus, and during cleavage and packaging. A comparison of the time course of replication of plasmids containing ori_S and two 300-bp homologous sequences, and the frequency of recombination between these sequences in HSV-1–infected Vero cells, have shown them to closely parallel each other during infection (303). The basis for the close parallelism is not known; however, a recombinase could be recruited to the replicating DNA either by the complex of replication enzymes or by changes in the DNA during replication. For example, replication introduces single-stranded breaks in the DNA, and such breaks are recombinogenic. Alternatively, the concatemeric structures that are generated during rolling-circle replication might be favored substrates for the recombination machinery (cellular and/or viral) (25, 26). In a recent study it was observed that although linearized plasmids were poorly replicated in HSV-1–infected cells, the introduction of directly repeated sequences at their ends greatly enhanced the efficiency of replication. Presumably, recombination between the repeated sequences resulted in the generation of circular molecules that are the required templates for replication (19; X Yao & P Elias, personal communication).

The inversion of the U_L and U_S elements of the HSV-1 genome, another recombinational event, has been investigated by means of an assay that detects

deletion of DNA segments flanked by directly repeated *a* sequences in plasmids transiently maintained in Vero cells. Recombination between the *a* sequences occurred at a high frequency (approximately 8%) in HSV-1–infected cells and at a much lower frequency ($<0.1\%$) in uninfected cells and in unreplicated plasmids in HSV-1–infected cells (303). With plasmids containing direct repeats of approximately the same size as the *a* sequence and with a wide range of $G + C$ content (22–73%), the recombination frequency was lower than with the 83% $G + C$ *a* sequence (32). The *a* sequence contains 20-bp direct repeats (DR1) at each end and two unique segments (U_b and U_c) separated by arrays of direct repeats (Figure 1). By replacing segments of the *a* sequence with DNA segments of similar length, it was found that the 95-bp U_c-DR1 segment is necessary for the enhanced recombination frequency observed with the *a* sequence (303). However, it is presently unclear whether the *a* sequence is the site at which a site-specific recombinase functions to promote the U_L-U_S inversions or is simply a hot spot for homologous recombination. It is, however, clear that HSV-1 DNA replication is specifically required for the high frequency of recombination observed both between *a* sequences and between other sequences of comparable size, because the recombination frequency in uninfected cells is one to two orders of magnitude lower than in HSV-1–infected cells (see above). A similarly low frequency of homologous recombination between *a* sequences is observed during replication from an SV40 origin in COS cells (African green monkey fibroblasts transformed with origin-defective SV40). In this case, a high frequency of recombination was seen; however, recombination was almost entirely nonhomologous (304).

In addition to its role in U_L-U_S sequence inversions, in the circularization of the linear HSV-1 genome prior to its replication, and in cleavage and packaging, recombination may also function directly in replication by generating single-stranded segments that can serve as primers for chain extension in a manner analogous to that observed during bacteriophage T4 DNA replication (305, 306). Another possibility is that a recombinational event during or following the theta phase of HSV-1 replication may constitute the switch from theta to rolling-circle DNA replication, analogous to what may occur in the lytic phase of bacteriophage λ replication (302).

> **Visit the *Annual Reviews* home page at**
> **http://www.annurev.org.**

Literature Cited

1. Fields BN, Knipe DM, Howley PM, eds. 1996. *Fields Virology*. Philadelphia: Lippincott-Raven
2. Roizman B, Sears AE. 1996. See Ref. 1, pp. 2231–95
3. Jenkins FJ, Roizman B. 1986. *J. Virol.* 29:448–57
4. Poffenberger KL, Tabares E, Roizman B. 1983. *Proc. Natl. Acad. Sci. USA* 80:2690–94
5. Stow ND. 1982. *EMBO J.* 1:863–67
6. Weller SK, Spadoro A, Schaffer JE, Murray AW, Maxam AM, Schaffer PA. 1985. *Mol. Cell. Biol.* 5:930–42
7. Roizman B, Aurelian L, Roane PR. 1963. *Virology* 21:482–98
8. Roizman B, Roane PR. 1964. *Virology* 22:262–69
9. Roizman B, Borman GS, Kamali-Rousta M. 1965. *Nature* 206:1374–75
10. Igarashi K, Fawl R, Roller RJ, Roizman B. 1993. *J. Virol.* 67:2123–32
11. Jacob RJ, Roizman B. 1977. *J. Virol.* 23:394–411
12. Becker Y, Asher Y, Weinberg-Zahlering E, Rabkin S, Friedmann A, Kessler E. 1978. *J. Gen. Virol.* 40:319–35
13. Jacob RJ, Morse LS, Roizman B. 1979. *J. Virol.* 29:448–57
14. Jongeneel CV, Bachenheimer SL. 1981. *J. Virol.* 30:656–60
15. Davison AJ, Wilkie NM. 1983. *J. Gen. Virol.* 64:1–18
16. Poffenberger KL, Roizman B. 1985. *J. Virol.* 53:587–95
17. Garber DA, Beverly SM, Coen DM. 1993. *Virology* 197:459–62
18. Severini A, Morgan AR, Tovell DR, Tyrrell DLJ. 1994. *Virology* 200:428–35
19. Umene K, Nishimoto T. 1996. *J. Gen. Virol.* 77:2261–70
20. Zhang X, Efstathiou S, Simmons A. 1994. *Virology* 202:530–39
21. Shlomai J, Friedmann A, Becker Y. 1976. *Virology* 69:647–59
22. Friedmann A, Shlomai J, Becker Y. 1977. *J. Gen. Virol.* 34:507–22
23. Hirsch I, Cabral G, Patterson M, Biswal N. 1977. *Virology* 81:48–61
24. Severini A, Scraba DG, Tyrrell DLJ. 1996. *J. Virol.* 70:3169–75
25. Rabkin SD, Hanlon B. 1990. *J. Virol.* 64:4957–67
26. Skaliter R, Lehman IR. 1994. *Proc. Natl. Acad. Sci. USA* 91:10665–69
27. Skaliter R, Makhov AM, Griffith JD, Lehman IR. 1996. *J. Virol.* 70:1132–36
28. Mocarski ES, Roizman B. 1982. *Cell* 31:89–97
29. Deiss LP, Chou J, Frenkel N. 1986. *J. Virol.* 59:605–18
30. Chou J, Roizman B. 1989. *J. Virol.* 63:1059–68
31. Wohlrab F, Chatterjee S, Wells RD. 1991. *Proc. Natl. Acad. Sci. USA* 88:6432–36
32. Dutch RE, Zemelman BV, Lehman IR. 1994. *J. Virol.* 68:3733–41
33. Bruckner RC, Dutch RE, Zemelman BV, Mocarski ES, Lehman IR. 1992. *Proc. Natl. Acad. Sci. USA* 89:10950–54
34. Addison C, Rixon FJ, Palfreyman JW, O'Hara M, Preston VG. 1984. *Virology* 138:246–59
35. Addison C, Rixon FJ, Preston VG. 1990. *J. Gen. Virol.* 71:2377–84
36. Al-Kobaisi MF, Rixon FJ, McDougall I, Preston VG. 1991. *Virology* 180:380–88
37. Sherman G, Bachenheimer SL. 1987. *Virology* 158:427–30
38. Sherman G, Bachenheimer SL. 1988. *Virology* 163:471–80
39. Tengelsen LA, Pederson NE, Shaver PR, Wathen MW, Homa FL. 1993. *J. Virol.* 67:3470–80
40. Desai P, DeLuca NA, Glorioso JC, Person S. 1993. *J. Virol.* 67:1357–64
41. Shao L, Rapp LM, Weller SK. 1993. *Virology* 196:146–62
42. Martinez R, Shao L, Bronstein JC, Weber PC, Weller SK. 1996. *Virology* 215:152–64
43. Martinez R, Sarisky RT, Weber PC, Weller SK. 1996. *J. Virol.* 70:2075–85
44. Schroder SH, Stegmann B, Laupe HF, Kaerner HC. 1975/1976. *Intervirology* 6:270–84
45. Frenkel N, Locker H, Batterson W, Hayward GS, Roizman B. 1976. *J. Virol.* 20:527–31
46. Kaerner HC, Maichle IB, Ott A, Schroder CH. 1979. *Nucleic Acids Res.* 6:1467–78
47. Frenkel N, Locker H, Vlazny DA. 1980. *Ann. NY Acad. Sci.* 354:347–70
48. Denniston KJ, Madden MJ, Enquist W, Van de Woude G. 1981. *Gene* 15:365–78
49. Vlazny DA, Frenkel N. 1981. *Proc. Natl. Acad. Sci. USA* 78:742–46
50. Cuifo DM, Hayward GS. 1981. In *Herpesvirus DNA*, ed. Y Becker, pp. 107–128. The Hague: Nijhoff
51. Kaerner HC, Ott-Hartmann A, Schatten R, Schroder CH, Gray CP. 1981. *J. Virol.* 39:75–81

52. Spaete RR, Frenkel N. 1982. *Cell* 30:295–304
53. Spaete RR, Frenkel N. 1985. *Proc. Natl. Acad. Sci. USA* 82:694–98
54. Mocarski ES, Roizman B. 1982. *Proc. Natl. Acad. Sci. USA* 79:5626–30
55. Stow ND, McMonagle EC. 1983. *Virology* 130:427–38
56. Wong SW, Schaffer PA. 1991. *J. Virol.* 65:2601–11
57. Hardwicke MA, Schaffer PA. 1995. *J. Virol.* 69:1377–88
58. Weir HM, Stow ND. 1990. *J. Gen. Virol.* 71:1379–85
59. Hernandez TR, Dutch RE, Lehman IR, Gustafsson C, Elias P. 1991. *J. Virol.* 65:1649–52
60. Martin DW, Deb SP, Klauer JS, Deb S. 1991. *J. Virol.* 65:4359–69
61. Longnecker R, Roizman B. 1986. *J. Virol.* 58:583–91
62. Polvino-Bodnar M, Orberg PK, Schaffer PA. 1987. *J. Virol.* 61:3528–35
63. Dabrowski CE, Schaffer PA. 1991. *J. Virol.* 65:3140–50
64. Dabrowski CE, Carmillo PJ, Schaffer PA. 1994. *Mol. Cell. Biol.* 14:2545–55
65. Sears AE, Roizman B. 1990. *Proc. Natl. Acad. Sci. USA* 87:9441–44
66. Brown SM, Ritchie DA, Subak-Sharpe JH. 1973. *J. Gen. Virol.* 18:329–46
67. Schaffer PA, Aron GM, Biswal N, Benyesh-Melnick M. 1973. *Virology* 53:57–71
68. Aron GM, Purifoy DJM, Schaffer PA. 1975. *J. Virol.* 16:498–507
69. Stow ND, Subak-Sharpe JH, Wilkie NM. 1978. *J. Virol.* 28:182–92
70. Parris DS, Courtney RJ, Schaffer PA. 1978. *Virology* 90:177–86
71. Chu CT, Parris DS, Dixon RAF, Farber FE, Schaffer PA. 1979. *Virology* 98:168–81
72. Parris DS, Dixon RAF, Schaffer PA. 1980. *Virology* 100:275–87
73. Weller SK, Aschman DP, Sacks WR, Coen DM, Schaffer PA. 1983. *Virology* 130:290–305
74. Weller SK, Lee KJ, Sabourin DJ, Schaffer PA. 1983. *J. Virol.* 45:354–66
75. Matz B, Subak-Sharpe JH, Preston VG. 1983. *J. Gen. Virol.* 64:2261–70
76. Hay J, Subak-Sharpe JH. 1976. *J. Gen. Virol.* 31:145–48
77. Chartrand P, Stow ND, Timbury MC, Wilkie NM. 1979. *J. Virol.* 31:265–76
78. Chartrand P, Crumpacker CS, Schaffer PA, Wilkie NM. 1980. *Virology* 103:311–25
79. Coen DM, Furman PA, Gelep PT, Schaffer PA. 1982. *J. Virol.* 41:909–18
80. Honess RW, Purifoy DJ, Young D, Gopal R, Cammack N, O'Hare P. 1984. *J. Gen. Virol.* 65:1–17
81. Coen DM, Ashman DP, Gelep PT, Retondo MJ, Weller SK, Schaffer PA. 1984. *J. Virol.* 49:236–47
82. Quinn JP, McGeoch DJ. 1985. *Nucleic Acids Res.* 13:8143–63
83. Bayliss GJ, Marsden HS, Hay J. 1975. *Virology* 68:124–34
84. Powell KL, Purifoy DJM. 1976. *Intervirology* 7:225–39
85. Purifoy DJ, Lewis RB, Powell KL. 1977. *Nature* 269:621–23
86. Conley AJ, Knipe DM, Jones PC, Roizman B. 1981. *J. Virol.* 37:191–206
87. Powell KL, Littler E, Purifoy DJM. 1981. *J. Virol.* 39:894–902
88. Purifoy DJM, Powell KL. 1981. *J. Gen. Virol.* 54:219–22
89. Challberg MD. 1986. *Proc. Natl. Acad. Sci. USA* 83:9094–98
90. Wu CA, Nelson NJ, McGeoch DJ, Challberg MD. 1988. *J. Virol.* 62:435–43
91. McGeoch DJ, Dalrymple MA, Dolan A, McNab D, Perry LJ, et al. 1988. *J. Virol.* 62:444–53
92. Zhu L, Weller SK. 1988. *Virology* 166:366–78
93. Zhu LA, Weller SK. 1992. *J. Virol.* 66:458–68
94. Zhu LA, Weller SK. 1992. *J. Virol.* 66:469–79
95. Carmichael EP, Weller SK. 1989. *J. Virol.* 63:591–99
96. Carmichael EP, Kosovsky M.J, Weller SK. 1988. *J. Virol.* 62:91–99
97. Malik AK, Martinez R, Muncy L, Carmichael EP, Weller SK. 1992. *Virology* 190:702–15
98. Johnson PA, Best MG, Friedmann T, Parris DS. 1991. *J. Virol.* 65:700–10
99. Goldstein DJ, Weller SK. 1988. *J. Virol.* 62:2970–77
100. Stow ND. 1992. *J. Gen. Virol.* 73:313–21
101. Elias P, O'Donnell ME, Mocarski ES, Lehman IR. 1986. *Proc. Natl. Acad. Sci. USA* 83:6322–26
102. Elias P, Lehman IR. 1988. *Proc. Natl. Acad. Sci. USA* 85:2959–63
103. Olivo PD, Nelson NJ, Challberg MD. 1988. *Proc. Natl. Acad. Sci. USA* 85:5414–18
104. McGeoch DJ, Dalrymple MA, Davison AJ, Dolan A, Frame MC, et al. 1988. *J. Gen. Virol.* 69:1531–74
105. Walker JE, Saraste M, Runswick MJ, Gay NJ. 1982. *EMBO J.* 1:945–51

106. Gorbalenya AE, Koonin EV, Donchenko AP, Blinov VM. 1989. *Nucleic Acids Res.* 17:4713–30
107. Martinez R, Shao L, Weller SK. 1992. *J. Virol.* 66:6735–46
108. Stow ND, Hammarsten O, Arbuckle MI, Elias P. 1993. *Virology* 196:413–18
109. Makhov AM, Boehmer PE, Lehman IR, Griffith JD. 1996. *EMBO J.* 15:1742–50
110. Makhov AM, Boehmer PE, Lehman IR, Griffith JD. 1996. *J. Mol. Biol.* 258:789–99
111. Bruckner RC, Crute JJ, Dodson MS, Lehman IR. 1991. *J. Biol. Chem.* 266:2669–74
112. Fierer DS, Challberg MD. 1992. *J. Virol.* 66:3986–95
113. Stabell EC, Olivo PD. 1993. *Nucleic Acids Res.* 21:5203–11
114. Fierer DS, Challberg MD. 1995. *J. Biol. Chem.* 270:7330–34
115. Elias P, Gustafsson CM, Hammarsten O, Stow ND. 1992. *J. Biol. Chem.* 267:17424–29
116. Hazuda DJ, Perry HC, McClements WL. 1992. *J. Biol. Chem.* 267:14309–15
117. Deb S, Deb SP. 1991. *J. Virol.* 65:2829–38
118. Weir HM, Calder JM, Stow ND. 1989. *Nucleic Acids Res.* 17:1409–25
119. Arbuckle MI, Stow ND. 1993. *J. Gen. Virol.* 74:1349–55
120. Martin DW, Munoz RM, Oliver D, Subler MA, Deb S. 1994. *Virology* 198:71–80
121. Stow ND. 1993. *Nucleic Acids Res.* 21:87–92
122. Perry HC, Hazuda DJ, McClements WL. 1993. *Virology* 193:73–79
123. Baradaran K, Dabrowski CE, Schaffer PA. 1994. *J. Virol.* 68:4251–61
124. Baradaran K, Hardwicke MA, Dabrowski CE, Schaffer PA. 1996. *J. Virol.* 70:5673–79
125. Koff A, Tegtmeyer P. 1988. *J. Virol.* 62:4096–103
126. Elias P, Gustafsson CM, Hammarsten O. 1990. *J. Biol. Chem.* 265:17167–73
127. Rabkin SD, Hanlon B 1991. *Proc. Natl. Acad. Sci. USA* 88:10946–50
128. Gustafsson CM, Hammarsten O, Falkenberg M, Elias P. 1994. *Proc. Natl. Acad. Sci. USA* 91:4629–33
129. Deb S, Deb SP. 1989. *Nucleic Acids Res.* 17:2733–52
130. Hazuda DJ, Perry HC, Naylor AM, McClements WL. 1991. *J. Biol. Chem.* 266:24621–26
131. Gustafsson CM, Falkenberg M, Simonsson S, Valadi H, Elias P. 1995. *J. Biol. Chem.* 270:19028–34
132. Dodson MS, Lehman IR. 1993. *J. Biol. Chem.* 268:1213–19
133. Boehmer PE, Dodson MS, Lehman IR. 1993. *J. Biol. Chem.* 268:1220–25
134. Earnshaw DL, Jarvest RL. 1994. *Biochem. Biophys. Res. Comm.* 199:1333–40
135. Villani G, Pillaire MJ, Boehmer PE. 1994. *J. Biol. Chem.* 269:21676–81
136. Borowiec JA. 1996. See Ref. 307, pp. 545–574.
137. Hassell JA, Brinton BT. 1996. See Ref. 307, pp. 639–678
138. Stenlund A. 1996. See Ref. 307, pp. 679–698.
139. Koff A, Schwedes JF, Tegtmeyer P. 1991. *J. Virol.* 65:3284–92
140. Berg JM. 1986. *Science* 232:485–87
141. Gao M, Bouchey J, Curtin K, Knipe DM. 1988. *Virology* 163:319–29
142. Wang YS, Hall J. 1990. *J. Virol.* 64:2082–89
143. Gupte SS, Olson JW, Ruyechan WT. 1991. *J. Biol. Chem.* 266:11413–16
144. Ruyechan WT. 1988. *J. Virol.* 62:810–17
145. Ruyechan WT, Olsen JW. 1992. *J. Virol.* 66:6273–79
146. Leinbach SS, Heath LS. 1989. *Biochem. Biophys. Acta* 1008:281–86
147. Ruyechan WT. 1983. *J. Virol.* 46:661–66
148. Lee CK, Knipe DM. 1985. *J. Virol.* 54:731–38
149. Ruyechan WT, Weir AC. 1984. *J. Virol.* 52:727–33
150. Boehmer PE, Lehman IR. 1993. *J. Virol.* 67:711–15
151. Dutch RE, Lehman IR. 1993. *J. Virol.* 67:6945–49
152. Hernandez TR, Lehman IR. 1990. *J. Biol. Chem.* 265:11227–32
153. O'Donnell ME, Elias P, Funnell BE, Lehman IR. 1987. *J. Biol. Chem.* 262:4260–66
154. Bruce CP, Pearson CK. 1983. *Biochem. Biophys. Res. Comm.* 116:327–34
155. Georgaki A, Strack B, Podust V, Hubscher U. 1992. *FEBS Lett.* 308:240–44
156. Georgaki A, Hubscher U. 1993. *Nucleic Acids Res.* 21:3659–65
157. Bortner C, Hernandez TR, Lehman IR, Griffith J. 1993. *J. Mol. Biol.* 231:241–50
158. Godowski PJ, Knipe DM. 1983. *J. Virol.* 47:478–86
159. Godowski PJ, Knipe DM. 1985. *J. Virol.* 55:357–65
160. Godowski PJ, Knipe DM. 1986. *Proc. Natl. Acad. Sci. USA* 83:256–60
161. Gao M, Knipe DM. 1991. *J. Virol.* 65:2666–75

162. Crute JJ, Mocarski ES, Lehman IR. 1988. *Nucleic Acids Res.* 16:6585–96

163. Crute JJ, Tsurumi T, Zhu LA, Weller SK, Olivo PD, et al. 1989. *Proc. Natl. Acad. Sci. USA* 86:2186–89

164. Sherman G, Gottlieb J, Challberg MD. 1992. *J. Virol.* 66:4884–92

165. Woodward-Graves KL, Weller SK. 1996. *J. Biol. Chem.* 271:13629–35

166. Klinedinst DK, Challberg MD. 1994. *J. Virol.* 68:3693–701

167. Dracheva S, Koonin EV, Crute JJ. 1995. *J. Biol. Chem.* 270:14148–53

168. Dodson MS, Crute JJ, Bruckner RC, Lehman IR. 1989. *J. Biol. Chem.* 264:20835–38

169. Crute JJ, Lehman IR. 1991. *J. Biol. Chem.* 266:4484–88

170. Calder JM, Stow ND. 1990. *Nucleic Acids Res.* 18:3573–78

171. Dodson MS, Lehman IR. 1991. *Proc. Natl. Acad. Sci. USA* 88:1105–9

172. Parry ME, Stow ND, Marsden HS. 1993. *J. Gen. Virol.* 74:607–12

173. Crute JJ, Bruckner RC, Dodson MS, Lehman IR. 1991. *J. Biol. Chem.* 266:21252–56

174. Tanguy Le Gac N, Villani G, Hoffman J-S, Boehmer PE. 1996. *J. Biol. Chem.* 271:21645–51

175. Tenney DJ, Hurlburt WW, Micheletti PA, Bifano M, Hamatake RK. 1994. *J. Biol. Chem.* 269:5030–35

176. Tenney DJ, Schaeffer AK, Hurlburt WW, Bifano M, Hamatake RK. 1995. *J. Biol. Chem.* 270:9129–36

177. Keir HM, Gold E. 1963. *Biochim. Biophys. Acta* 72:263–76

178. Keir HM, Subak-Sharpe H, Shedden WI, Watson DH, Wildy P. 1966. *Virology* 30:154–57

179. Knopf KW. 1979. *Eur. J. Biochem.* 98:231–44

180. Powell KL, Purifoy DJ. 1977. *J. Virol.* 24:618–26

181. Gibbs JS, Chiou HC, Hall JD, Mount DW, Retondo MJ, et al. 1985. *Proc. Natl. Acad. Sci. USA* 82:7969–73

182. Hall JD, Gibbs JS, Coen DM, Mount DW. 1986. *DNA* 5:281–88

183. Digard P, Coen DM. 1990. *J. Biol. Chem.* 265:17393–96

184. Blanco L, Bernad A, Blasco MA, Salas M. 1991. *Gene* 100:27–38

185. Hall JD, Orth KL, Ser KL, Swihart BM, Senese RA. 1995. *J. Gen. Virol.* 76:2999–3008

186. Vaughan PJ, Purifoy DJM, Powell KL. 1985. *J. Virol.* 53:501–8

187. Gallo ML, Jackwood DH, Murphy M, Marsden HS, Parris DS. 1988. *J. Virol.* 62:2874–83

188. Crute JJ, Lehman IR. 1989. *J. Biol. Chem.* 264:19266–70

189. Parris DS, Cross A, Haarr L, Orr A, Frame MC, et al. 1988. *J. Virol.* 62:818–25

190. Hamatake RK, Bifano M, Tenney DJ, Hurlburt WW, Cordingley MG. 1993. *J. Gen. Virol.* 74:2181–89

191. Digard P, Bebrin WR, Weisshart K, Coen DM. 1993. *J. Virol.* 67:398–406

192. Digard P, Chow CS, Pirrit L, Coen DM. 1993. *J. Virol.* 67:1159–68

193. Reddig PJ, Grinstead LA, Monahan SJ, Johnson PA, Parris DS. 1994. *Virology* 200:447–56

194. Marsden HS, Murphy M, McVey GL, MacEachran KA, Owsianka AM, Stow ND. 1994. *J. Gen. Virol.* 75:3127–35

195. Digard P, Williams KP, Hensley P, Brooks IS, Dahl CE, Coen DM. 1995. *Proc. Natl. Acad. Sci. USA* 92:1456–60

196. Ostrander M, Cheng YC. 1980. *Biochim. Biophys. Acta* 609:232–45

197. O'Donnell ME, Elias P, Lehman IR. 1987. *J. Biol. Chem.* 262:4252–59

198. Weissbach A, Hong SCL, Aucker J, Muller R. 1973. *J. Biol. Chem.* 248:6270–77

199. Wallace HM, Baybutt HN, Pearson CK, Keir HM. 1980. *J. Gen. Virol.* 49:397–400

200. Derse D, Cheng YC. 1981. *J. Biol. Chem.* 256:8525–30

201. Mao JC, Robishaw EE. 1975. *Biochemistry* 14:5475–79

202. Abbotts J, Nishiyama Y, Yoshida S, Loeb LA. 1987. *Nucleic Acids Res.* 15:1185–98

203. Marcy AI, Olivo P, Challberg MD, Coen DM. 1990. *Nucleic Acids Res.* 18:1207–15

204. Weisshart K, Kuo AA, Hwang CBC, Kumura K, Coen DM. 1994. *J. Biol. Chem.* 269:22788–96

205. Hall JD, Orth KL, Claus-Walker D. 1996. *J. Virol.* 70:4816–18

206. Derbyshire V, Grindley NDF, Joyce CM. 1991. *EMBO J.* 10:17–24

207. Gibbs JS, Weisshart K, Digard P, de Bruyn Kops A, Knipe DM, Coen DM. 1991. *Mol. Cell. Biol.* 11:4786–95

208. Knopf CW, Weisshart K. 1990. *Eur. J. Biochem.* 191:263–73

209. Dorsky DI, Crumpacker CS. 1988. *J. Virol.* 62:3224–32

210. Haffey ML, Novotny J, Bruccoleri RE, Carroll RD, Stevens JT, Mathews JT. 1990. *J. Virol.* 64:5008–18

211. Knopf CW, Weisshart K. 1988. *Biochim. Biophys. Acta* 951:298–314
212. Weisshart K, Knopf CW. 1988. *Eur. J. Biochem.* 174:707–16
213. Gallo ML, Dorsky DI, Crumpacker CS, Parris DS. 1989. *J. Virol.* 63:5023–29
214. Gottlieb J, Marcy AI, Coen DM, Challberg MD. 1990. *J. Virol.* 64:5976–87
215. Stukenberg PT, Studwell-Vaughn PS, O'Donnell ME. 1991. *J. Biol. Chem.* 266:11328–34
216. Kong XP, Onrust R, O'Donnell ME, Kuriyan J. 1992. *Cell* 69:425–37
217. Hubscher U, Maga G, Podust VN. 1996. See Ref. 307, pp. 525–544
218. Kuriyan J, O'Donnell M. 1993. *J. Mol. Biol.* 234:915–25
219. Chow CS, Coen DM. 1995. *J. Virol.* 69:6965–71
220. Gottlieb J, Challberg MD. 1994. *J. Virol.* 68:4937–45
221. Owsianka AM, Hart G, Murphy M, Gottlieb J, Boehme R, et al. 1993. *J. Virol.* 67:258–64
221a. Gao M, DiTusa SF, Cordingley MG. 1993. *Virology* 194:647–53
222. Monahan SJ, Barlam TF, Crumpacker CS, Parris DS. 1993. *J. Virol.* 67:5922–31
223. Tenney DJ, Hurlburt WW, Bifano M, Stevens JT, Micheletti PA, et al. 1993. *J. Virol.* 67:1959–66
224. Coen DM, Furman PA, Ashman DP, Schaffer PA. 1983. *Nucleic Acids Res.* 11:5287–97
225. Hall JD, Wang Y, Pierpont J, Berlin MS, Rundlett SE, Woodward S. 1989. *Nucleic Acids Res.* 17:9231–44
226. Hall JD, Woodward S. 1989. *J. Virol.* 63:2874–76
227. Wang YS, Woodward S, Hall JD. 1992. *J. Virol.* 66:1814–16
228. Coen DM, Schaffer PA. 1980. *Proc. Natl. Acad. Sci. USA* 77:2265–69
229. Schnipper LE, Crumpacker CS. 1980. *Proc. Natl. Acad. Sci. USA* 77:2270–73
230. Field HJ, Darby G, Wildy P. 1980. *J. Gen. Virol.* 49:115–24
231. Knopf KW, Kaufman ER, Crumpacker C. 1981. *J. Virol.* 39:746–57
232. Furman PA, Coen DM, Sinclair MH, Schaffer PA. 1981. *J. Virol.* 40:936–41
233. Honess RW, Watson DH. 1977. *J. Virol.* 21:584–600
234. Jofre JT, Schaffer PA, Parris DS. 1977. *J. Virol.* 23:833–36
235. Knopf CW. 1987. *J. Gen. Virol.* 68:1429–33
236. Larder BA, Kemp SD, Darby G. 1987. *EMBO J.* 6:169–75
237. Gibbs JS, Chiou HC, Bastow KF, Cheng YC, Coen DM. 1988. *Proc. Natl. Acad. Sci. USA* 85:6672–76
238. Mathews JT, Carroll RD, Stevens JT, Haffey ML. 1989. *J. Virol.* 63:4913–18
239. Hwang CB, Ruffner KL, Coen DM. 1992. *J. Virol.* 66:1774–76
240. Hall JD, Coen DM, Fisher BL, Weisslitz M, Rall S, et al. 1984. *Virology* 132:26–37
241. Hall JD, Furman PA, Sinclair MH, Knopf CW. 1985. *Proc. Natl. Acad. Sci. USA* 82:3889–93
242. Boehmer PE, Lehman IR. 1993. *Proc. Natl. Acad. Sci. USA* 90:8444–48
243. Boehmer PE, Craigie MC, Stow ND, Lehman IR. 1994. *J. Biol. Chem.* 269:29329–34
244. McLean GW, Abbotts AP, Parry ME, Marsden HS, Stow ND. 1994. *J. Gen. Virol.* 75:2699–706
245. Lee SS, Dong Q, Wang TS, Lehman IR. 1995. *Proc. Natl. Acad. Sci. USA* 92:7882–86
246. Sheaff R, Ilsley D, Kuchta R. 1991. *Biochemistry* 30:8590–97
247. Kuchta RD, Willhelm L. 1991. *Biochemistry* 30:797–803
248. Littler E, Purifoy D, Minson A, Powell KL. 1983. *J. Gen. Virol.* 64:983–95
249. Vaughan PJ, Banks LM, Purifoy DJM, Powell KL. 1984. *J. Gen. Virol.* 65:2033–41
250. Chiou HC, Weller SK, Coen DM. 1985. *Virology* 145:213–26
251. Kornberg A, Baker T. 1992. DNA *Replication*. New York: Freeman
252. Biswal N, Feldan P, Levy CC. 1983. *Biochim. Biophys. Acta* 740:379–89
253. Bapat AR, Han FS, Liu ZC, Zhou BS, Cheng YC. 1987. *J. Gen. Virol.* 68:2231–37
254. Ebert SN, Subramanian D, Shtrom SS, Chung IK, Parris DS, Muller MT. 1994. *J. Virol.* 68:1010–20
255. Hammarsten O, Yao X, Elias P. 1996. *J. Virol.* 70:4523–29
256. Hafner J, Mohammad F, Farber FE. 1987. *Biochem. Biophys. Acta* 910:85–88
257. Thomas MS, Banks LM, Purifoy DJM, Powell KL 1988. *J. Virol.* 62:1550–57
258. Thomas MS, Gao M, Knipe DM, Powell KL. 1992. *J. Virol.* 66:1152–61
259. Quinlan MP, Chen LB, Knipe DM. 1984. *Cell* 36:857–68
260. Gao M, Knipe DM. 1989. *J. Virol.* 63:5258–67
261. Olivo PD, Nelson NJ, Challberg MD. 1989. *J. Virol.* 63:196–204

262. Bush M, Yager DR, Gao M, Weisshart K, Marcy AI, et al. 1991. *J. Virol.* 65:1082–89

263. Gao M, Knipe DM. 1992. *Mol. Cell. Biol.* 12:1330–39

264. Calder JM, Stow EC, Stow ND. 1992. *J. Gen. Virol.* 73:531–38

265. Marsden HS, Cross AM, Francis GJ, Patel AH, MacEachran K, et al. 1996. *J. Gen. Virol.* 77:2241–49

266. de Bruyn Kops A, Knipe DM. 1988. *Cell* 55:857–68

267. Lukonis CJ, Weller SK. 1996. *J. Virol.* 70:1751–58

268. Liptak LM, Uprichard SL, Knipe DM. 1996. *J. Virol.* 70:1759–67

269. Goodrich LD, Schaffer PA, Dorsky DI, Crumpacker CS, Parris DS. 1990. *J. Virol.* 64:5738–49

270. Wilcock D, Lane DP. 1991. *Nature* 349:429–31

271. Banks L, Purifoy DJM, Hurst PF, Killington RA, Powell KL. 1983. *J. Gen. Virol.* 64:2249–60

272. Friedberg EC, Walker GC, Siede W. 1995. DNA *Repair Mutagenesis*. Washington, DC: ASM

273. Caradonna SJ, Cheng YC. 1981. *J. Biol. Chem.* 256:9834–37

274. Caradonna SJ, Worrad D, Lirette R. 1987. *J. Virol.* 61:3040–47

275. Argnani R, Focher F, Zucchini S, Verri A, Wright GE, et al. 1995. *Virology* 211:307–11

276. Olsen LC, Aasl R, Wittwer CU, Krokan HE, Hell DE. 1989. *EMBO J.* 8:3121–25

277. Savva R, McAuley-Hecht K, Brown T, Pearl L. 1995. *Nature* 373:487–93

278. Pyles RB, Thompson RL. 1994. *J. Virol.* 68:4963–72

279. Williams MV. 1984. *J. Biol. Chem.* 259:10080–84

280. Caradonna SJ, Adamkiewicz DM. 1984. *J. Biol. Chem.* 259:5459–64

281. Bjornberg O, Bergman AC, Rosengren AM, Persson R, Lehman IR, Nyman PO. 1993. *Prot. Expr. Purif.* 4:149–59

282. Fisher FB, Preston VG. 1986. *Virology* 148:190–97

283. Pyles RB, Sawtell NM, Thompson RL. 1992. *J. Virol.* 66:6706–13

284. Chen MS, Summers WP, Walker J, Summers WC, Prusoff WH. 1979. *J. Virol.* 30:942–45

285. Chen MS, Prusoff WH. 1978. *J. Biol. Chem.* 253:1325–27

286. Brown DG, Visse R, Shu G, Davies A, Rizkallah PJ, et al. 1995. *Nature Struc. Biol.* 2:876–81

287. Reardon JE, Spector T. 1989. *J. Biol. Chem.* 264:7405–12

288. Coen DM, Kosz-Vnenchak M, Jacobson JG, Leib DA, Bogard CL, et al. 1989. *Proc. Natl. Acad. Sci. USA* 86:4736–30

289. Bacchetti S, Evelegh MJ, Muirhead B. 1986. *J. Virol.* 57:1177–81

290. Ingemarson R, Lankinen H. 1987. *Virology* 56:417–22

291. Filatov D, Ingemarson R, Graslund A, Theler L. 1992. *J. Biol. Chem.* 267:15816–22

292. Ingemarson R, Graslund A, Darling A, Theler L. 1989. *J. Virol.* 63:3769–76

293. Cooper J, Conner J, Clements JB. 1995. *J. Virol.* 69:4979–85

294. Ali MA. 1995. *Virology* 207:409–16

295. Darling AJ. 1988. *J. Gen. Virol.* 69:515–23

296. Averett DR, Lubbers C, Elion GB, Spector T. 1983. *J. Biol. Chem.* 258:9831–38

297. Cosentino G, Lavallee P, Rakhit S, Plante R, Gaudette Y, et al. 1991. *Biochem. Cell Biol.* 69:79–83

298. Moss N, Deziel R, Adams J, Aubry N, Bailey M, et al. 1993. *J. Med. Chem.* 36:3005–9

299. Goldstein DJ, Weller SK. 1988. *J. Virol.* 62:196–205

300. Idowu AD, Fraser-Smith EB, Poffenberger KL, Herman RC. 1992. *Antiviral. Res.* 17:145–56

301. Ben-Porat T, Blankenship ML, DeMarchi JM, Kaplan AS. 1977. *J. Virology* 22:734–41

302. Stahl FW, Stahl MM. 1971. In *The Bacteriophage Lambda*, ed. AD Hershey. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press

303. Dutch RE, Bruckner RC, Mocarski ES, Lehman IR. 1992. *J. Virol.* 66:277–85

304. Dutch RE, Bianchi V, Lehman IR. 1995. *J. Virol.* 69:3084–89

305. Formosa T, Alberts BM. 1986. *Cell* 47:793–96

306. Mosig G, Eiserling F. 1988. In *The Bacteriophages*, ed. R Calendar, pp. 521–532. New York: Plenum

307. DePamphilis ML, ed. 1996. *DNA Replication in Eukaryotic Cells*. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press