25

Herpesvirus DNA Replication

Mark Challberg
Laboratory of Viral Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20892

The genomes of herpesviruses are linear double-stranded DNA molecules ranging in size from 120 kb to more than 200 kb. Although the many different herpesviruses display a wide variety of tissue tropisms and vary enormously in the way in which they interact with their natural hosts, one common feature of the biology of all herpesviruses is the mechanism by which they replicate their genomes during the lytic phase of the replication cycle. Lytic DNA replication in every herpesvirus studied occurs by a mechanism that generates long head-to-tail concatemers of viral genomes that are cleaved to unit-length genomes during the process of encapsidation. This common mode of lytic DNA replication reflects a conserved set of viral genes encoding the basic components of the replication machinery. Another common feature of herpesvirus biology is the capacity to remain latent in the infected host, but, unlike the case with lytic DNA replication, the mechanism by which the viral genomes are maintained during latency apparently differs considerably among the herpesviruses. The cells that harbor latent genomes are different for the different viruses, and perhaps the more intimate relationship between viral and host chromosomal replication during latency accounts for the greater diversity of mechanism. For example, Epstein-Barr virus (EBV), which is latent in dividing B cells, is replicated during latency from a latency-specific origin called *oriP* that is distinct from the origin of lytic DNA replication. Replication in this system requires a single virus-encoded protein (EBNA1) and is apparently carried out by the chromosomal replication machinery (see Yates, this volume). On the other hand, herpes simplex virus (HSV) is latent in postmitotic neurons, and there is no evidence for any viral DNA replication during latency. In this review, I focus on lytic DNA replication, with an emphasis on studies of HSV DNA replication, the herpesvirus system about which most is known.

DNA Replication in Eukaryotic Cells

MODE OF REPLICATION—IN VIVO STUDIES

The general picture of herpesvirus lytic replication derives from analyses of replication intermediates, which have two characteristic properties. First, DNA pulse-labeled in vivo with [^{3}H]thymidine sediments more rapidly than unit length viral DNA (Jacob and Roizman 1977). Second, pulse-labeled DNA is "endless"; i.e., the molecular termini of mature viral DNA are fused together (Jacob et al. 1979; Jongeneel and Bachenheimer 1981). On the basis of these two observations, it has been proposed that parental linear viral DNA is circularized shortly after entry into the host cell and that replication takes place predominantly by a rolling-circle mechanism, generating linear concatemers of tandemly repeated viral genomes (Jacob et al. 1979). In support of this model is the finding that circularization of input HSV genomes does take place, by a mechanism apparently involving direct ligation of the termini (Mocarski and Roizman 1982; Poffenberger and Roizman 1985; Garber et al. 1993). Since circularization occurs in the absence of viral gene expression, one of the host-cell ligases is likely to be responsible. Circularization of parental viral genomes prior to the onset of DNA synthesis is the simplest explanation for the complete lack of genomic termini in replicating DNA, and it seems likely that replication involves a rolling-circle intermediate. Nevertheless, recent experiments utilizing pulsed-field gel electrophoresis suggest that both the structure of replication intermediates and the replication process itself may be more complex than a simple rolling-circle model would predict (Bataille and Epstein 1994; McVoy and Adler 1994; Severini et al. 1994; Zhang et al. 1994). A number of investigators have observed that newly replicated DNA has a very slow electrophoretic mobility when analyzed by pulsed-field gel electrophoresis—the majority of nascent DNA will not enter a gel unless it is highly fragmented. This low electrophoretic mobility is partially retained even after digestion of the DNA with a restriction enzyme that cuts once per unit-length genome (Severini et al. 1994). These results, in conjunction with earlier electron microscopic studies (Jacob and Roizman 1977), suggest that newly replicated viral DNA is composed of highly branched, complex networks. The origin of these branched structures is not known, although the most plausible hypothesis is that they are derived at least in part by homologous recombination. Several studies have shown that HSV DNA in infected cells undergoes high levels of homologous recombination (Schaffer et al. 1974; Honess et al. 1980; Smiley et al. 1980) and that this high rate of recombination is closely linked to DNA synthesis (Schaffer et al. 1974; Honess et al. 1980; Smiley et al. 1980; Weber et al. 1988, 1990; Dutch et al. 1992, 1995). By analogy with bacteriophage T4,

which produces replication intermediates with very similar properties, it is possible that the networks are formed by a combination of DNA synthesis and recombination and that resolution of recombination intermediates is a slow step. There are at least two key questions that need to be answered: (1) Is recombination an important mechanism for generating replication forks once DNA replication is under way? and (2) What is the mechanism by which DNA replication and recombination are linked? It is possible that replication intermediates are inherently recombinogenic due to unligated strands or partially single-stranded regions. On the other hand, it is also possible that one (or more) virus-encoded protein acts as a recombinase. Although there is no biochemical evidence that suggests the existence of a herpesvirus-encoded recombinase, the virus-encoded single-stranded DNA-binding protein promotes the annealing of single strands (Bortner et al. 1993; Dutch and Lehman 1993), and the existence of a recombinase clearly cannot yet be ruled out.

ORIGINS OF DNA REPLICATION

The existence of *cis*-acting replication origins was first inferred from the structure of defective genomes (Frenkel et al. 1975, 1976; Schroder et al. 1975) that arise during serial passage of HSV at high multiplicities of infection. Plasmids containing origin sequences derived either from these defective genomes or from wild-type genomes are amplified when introduced into HSV-infected cells by transfection, and this transient plasmid amplification assay has been utilized both to identify the lytic origins of a number of other herpesviruses and as the basis for functional analyses of cloned origin sequences. Although, as noted above, the overall mode of lytic DNA replication is conserved among the herpesviruses, the sequence and structure of origins vary considerably among the various herpesvirus groups; by implication, therefore, it is likely that the mechanism of replication initiation varies as well. Schematic models of the lytic origins of HSV and human cytomegalovirus (HCMV) are presented in Figure 1, and their structure and function are described in more detail below. (The structure and function of the EBV lytic origin are reviewed by Yates, this volume.) These three viruses represent examples of all three major herpesvirus groups. It is readily apparent that there is no common arrangement of structural or functional elements, and the lack of similarity is underscored further by the differences in the virus-encoded proteins that interact with their cognate origins. As discussed below, HSV encodes a protein, UL9, that appears to be a classic initiator protein: It binds specifically to the HSV origins of replication and is a

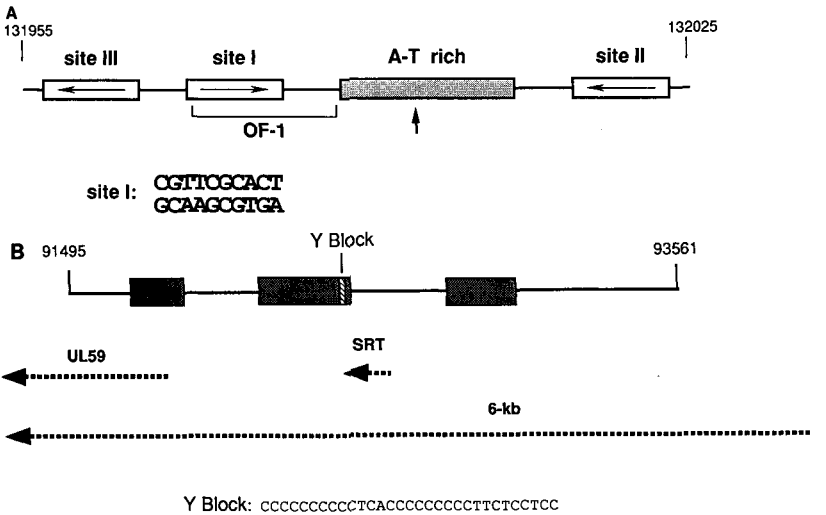

Figure 1 (*A*) Functional domains of the core *ori*$_S$ of HSV. See text for details. (*B*) Functional domains of the core *oriLyt* of HCMV (D. Anders, pers. comm.). The shaded areas represent regions into which insertion of a kanamycin-resistance cassette causes a >50-fold reduction in origin function in transfection assays. The dashed lines represent RNAs that either begin in or extend into the core origin region; SRT is a family of short transcripts that terminate within the Y block. See text for details.

helicase. On the other hand, the EBV-encoded protein that binds to the EBV *oriLyt* is the transcriptional activator protein BZLF1, a key regulatory protein that controls the switch from the latent to lytic transcription program and a protein with no known helicase activity. The CMV *oriLyt* has a number of possible sites for binding of cellular transcription factors, but no virus-encoded proteins that interact with the origin have been identified. The one feature that all of the herpesvirus origins do have in common is the requirement for the binding of transcriptional activator proteins for optimal origin activity. As discussed elsewhere in this volume and below, this is a feature that herpesvirus origins have in common with many eukaryotic origins. The mechanism(s) by which transcription factors enhance replication efficiency is not yet known.

HSV *ori*$_S$ and *ori*$_L$

The HSV genome contains two classes of origin sequences: *ori*$_S$, located in the inverted repeat segment flanking the short component of the

genome (and therefore present in the genome in two copies) and ori_L, the sequence of which comprises a 144-bp perfect palindrome, located between the *ul29* (single-stranded DNA-binding protein) and the *ul30* (*pol*) genes near the middle of the long unique component of the genome (Spaete and Frenkel 1982; Stow 1982; Vlasny and Frenkel 1982; Stow and McMonagle 1983; Weller et al. 1985). The sequences of ori_S and ori_L are closely related (Murchie and McGeoch 1982; Weller et al. 1985; Knopf et al. 1986). Both contain an extensive inverted repeat sequence, the central 18 bp of which are exclusively AT base pairs. As described below, the minimum sequences required for the function of ori_S correspond well to the region of highest similarity with ori_L (Lockshon and Galloway 1988). The binding sites for viral and cellular proteins have been shown to be equivalent, and, in transfection assays at least, ori_S and ori_L behave similarly (Hardwicke and Schaffer 1995). The significance of having three origins of replication in the HSV genome is not clear. Mutant viruses lacking ori_L or one or both copies of ori_S have been isolated and have no obvious growth defect either in cultured cells or in animal models (Poffenberger and Roizman 1985; Polvino-Bodnar et al. 1987; Igarashi et al. 1993). A virus lacking ori_L and containing two partially defective copies of ori_S has been constructed and has been shown to have a significant growth defect, whereas an analogous virus containing a wild-type ori_L has no growth defect (S. Wong and P. Schaffer, pers. comm.). These data constitute the strongest genetic evidence to date that ori_S and ori_L serve as essential *cis*-acting functions during the replication of viral DNA in vivo and provide additional support for the idea that ori_S and ori_L are at least partially redundant functionally. Recent studies have shown that the few nucleotide differences between the core regions of ori_S and ori_L result in the presence of a glucocorticoid response element that is not present in ori_S. Preliminary experiments suggest that in cells of neural origin, the presence of this binding site is responsible for a glucocorticoid-induced increase in efficiency of replication from ori_L, and its absence results in a glucocorticoid-induced decrease in efficiency from ori_S (M. Hardwicke and P. Schaffer, pers. comm.). Thus, it is possible that ori_L may have an important role in the initial rounds of DNA replication that occur upon the reactivation of latent genomes. It is clear, however, that ori_L is not absolutely required for reactivation (Polvino-Bodnar et al. 1987). Additional experiments will be required to determine the precise role of multiple origin sequences and to answer the question of whether initiation occurs predominantly at one class of origin sequence during particular phases of replication.

Several laboratories have carried out mutational analyses of plasmids containing ori_S, with similar results (Lockshon and Galloway 1988; Deb and Deb 1989; Weir and Stow 1990; Hernandez et al. 1991). ori_S can be divided into two components: the core origin containing UL9-binding sites, and flanking sequences that increase the efficiency of replication by 50-fold or more (Wong and Schaffer 1991). As mentioned, the minimal core origin sequence corresponds well to the region of highest similarity with ori_L. The flanking sequences contain a number of consensus binding sites for transcriptional activator proteins such as SP1 and NFI, and the available evidence suggests that the binding of one or more of these transcriptional regulators to sites in close juxtaposition to the core sequence may be critical for enhancing DNA replication.

The core origin sequence is composed of at least five functional domains: two high-affinity UL9-binding sites (called site I and site II or box I and box II) (Elias and Lehman 1988; Olivo et al. 1988; Weir et al. 1989; Elias et al. 1990); an AT-rich region; a sequence homologous to site I, but with much lower affinity for UL9, called site III (or box III) (Elias et al. 1990; Dabrowski and Schaffer 1991); and a binding site for an as-yet-uncharacterized cellular protein(s) called OF-1 (Dabrowski and Schaffer 1991; Dabrowski et al. 1994). Sites I and II are located on the arms of an imperfect 46-bp palindrome, separated by the AT-rich region. Site III and site I form the arms of another, shorter palindrome, with site III present just 5′ of site I. Nuclease protection, chemical modification, and saturation mutagenesis studies (Koff and Tegtmeyer 1988; Elias et al. 1990; Hazuda et al. 1991) have shown that the high-affinity recognition sequence for UL9 is contained in the sequence 5′-CGTTCGCACT. In ori_S (but not in ori_L), site II differs from site I at two positions, resulting in a reduced binding affinity for UL9 to about one-fifth that of site I (Elias and Lehman 1988; Elias et al. 1990; Hazuda et al. 1991). Site III differs in sequence from site I at only one position, but its affinity for UL9 is reduced to less that one-thousandth that of site I (Hazuda et al. 1991). The sequence of ori_L comprises a perfect palindrome (Weller et al. 1985). As a consequence of the greater symmetry in ori_L relative to ori_S, the two high-affinity binding sites (equivalent to site I in ori_S) for UL9 are identical, and ori_L contains two, rather than one, low-affinity binding sites (equivalent to site III in ori_S). The functional consequences of these differences, if any, are not known.

Genetic experiments indicate that these five domains of the core origin sequence are essential for optimal efficiency in promoting DNA replication in transient assays (Lockshon and Galloway 1988; Weir and Stow 1990; Hernandez et al. 1991). Mutations in site I or site II that

abolish UL9 binding eliminate or greatly reduce the replication efficiency of plasmids containing these sequences, and mutations in site III reduce replication efficiency by a factor of 5. Deletions or substitution of GC pairs for AT pairs in the central AT-rich region significantly decreases replication efficiency (Lockshon and Galloway 1988; Werstuck et al. 1990). Finally, mutations that eliminate OF-1 binding also diminish the replication efficiency of the origin-containing plasmids (Dabrowski et al. 1994).

Both ori_S and ori_L are located between divergently transcribed genes, and, as mentioned above, there is evidence that the binding of one or more transcriptional activator proteins to enhancer sequences flanking the core origin sequence does have a pronounced stimulatory effect on the extent of DNA replication (Wong and Schaffer 1991). In this regard, HSV DNA replication is similar to other better-characterized viral replication systems such as the adenoviruses and the papovaviruses. In the case of HSV, there is almost no information on the mechanism by which these stimulatory factors act. It seems unlikely that the stimulatory effect depends on transcription per se, since the stimulatory sequences do not include the known start sites for transcription of the surrounding genes, although this point will have to be more closely investigated in the future. In this context, it would be of some interest to know whether there is a difference in the time following infection at which initiation takes place at ori_S and ori_L. ori_S is flanked by the immediate-early class of genes, that is, genes that are transcribed immediately from the infecting viral genome, whereas ori_L is flanked by two delayed-early genes, the transcription of which depends on the presence of one or more immediate-early gene products. There is a transcript in HSV-infected cells that extends through ori_S (Hubenthal-Vass et al. 1987). Neither the 5′ nor 3′ end of this transcript is located within sequences that have an effect on origin function. It has been suggested that this transcript may play a role in regulating DNA replication, but there is no evidence in support of this speculation. Finally, since HSV DNA is thought not to be complexed with cellular histones following uncoating in the nucleus (Leinbach and Summers 1980), it seems unlikely that the role of transcription factors in DNA replication is to exclude the formation of nucleosomes in the vicinity of the origin, as has been suggested in the case of SV40 (Cheng and Kelly 1989). At this point, the available data seem to be most consistent with a model in which the binding of transcription factors near the origin stimulates the binding of critical replication factors to the origin, much as has been proposed for the role of NFI in adenovirus DNA replication. This model will be testable once more

information is gained concerning the events that occur at the core origin sequence.

HCMV *oriLyt*

The origin of HCMV lytic DNA replication was identified by a novel method utilizing an inhibitor of the HCMV DNA polymerase, ganciclovir, to terminate DNA synthesis shortly after initiation (Hamzeh et al. 1990), as well as by the more conventional approach of transient transfection assay (Anders and Punturieri 1991; Masse et al. 1992). Deletion analysis of the cloned *oriLyt* has shown that the core functional unit spans more than 2.0 kb of DNA near the middle of the U_L region of the viral genome (Anders et al. 1992; Y. Zhu and D. Anders, pers. comm.). As with HSV ori_S, the sequences flanking the core origin also contribute to the overall efficiency of replication. The structure of the 2.0-kb core origin is highly complex. The sequence contains a number of repeated elements, some of which include transcription factor binding motifs, as well as regions of dyad symmetry. Extensive mutational analysis of this sequence has revealed several structural elements that are critical for function, but the only non-repeated element that is absolutely required is an oligopyrimidine stretch (on the top strand) named the Y block (see Fig. 1B). This sequence motif is similar to the "downstream region" in the *oriLyt* of EBV (Schepers et al. 1993b) and is conserved in simian and murine CMV. As noted earlier, no HCMV-encoded protein has yet been shown to bind specifically to the *oriLyt* sequence. Recently, however, a family of short, non-polyadenylated transcripts (named the "short replicator transcripts"; SRT) expressed within *oriLyt* have been identified and characterized (L. Huang et al., pers. comm.). The 5′ ends of these RNAs are located about 200 bp to the right of the Y block (at position 92685), and the 3′ ends all map within or just to the left of the Y block. A very similar arrangement of transcript and oligopyrimidine sequence occurs in the mitochondrial heavy-strand replicator (Xu and Clayton 1995; see Clayton, this volume). In mitochondrial DNA, the oligopyrimidine sequence inhibits the release of the nascent transcript and promotes the formation of a displacement loop; the RNA is then processed and used as a primer for DNA synthesis. On the basis of this similarity, it has been suggested that a critical specific step in the initiation of CMV replication may be the synthesis of the SRT rather than, or in addition to, the site-specific binding of virus-encoded protein (D. Anders, pers. comm.). According to this model, the SRT either would act directly as a primer for DNA synthesis or would mediate a local unwind-

ing of the DNA, which would then serve as an entry point for the virus-encoded replication machinery. This model awaits experimental verification.

VIRAL REPLICATION PROTEINS

The complete set of viral genes that are required for HSV DNA replication were identified by means of a transient complementation assay in which cloned segments of HSV DNA were tested for the ability to support the replication of a cotransfected plasmid containing ori_S or ori_L (Challberg 1986; Wu et al. 1988). Seven genes were found to be both necessary and sufficient for origin-dependent DNA replication. The results of the transfection assay are supported by detailed mapping of available *ts* mutants with clear DNA-negative phenotypes and by the construction of viruses containing targeted null mutations (insertions or deletions) in these seven genes (Purifoy et al. 1977; Chartrand et al. 1980; Conley et al. 1981; Purifoy and Powell 1981; Coen et al. 1984; Orberg and Schaffer 1987; Weller et al. 1987; Carmichael et al. 1988; Goldstein and Weller 1988; Marchetti et al. 1988; Zhu and Weller 1988, 1992a; Carmichael and Weller 1989; Marcy et al. 1990a; Yamada et al. 1990; Johnson et al. 1991). The success of the systematic transfection assay in locating the essential replication genes in HSV has now been repeated for both HCMV (Pari and Anders 1993; Pari et al. 1993) and EBV (Fixman et al. 1992, 1995). The results of these studies, which are summarized in Table 1, as well as nucleotide sequence analyses of a number of other herpesviruses, lead to the following general conclusions. The genes required for lytic DNA replication include a set of six genes that are conserved in all herpesviruses. As discussed in detail below, the products of these six genes in HSV (UL5, UL8, UL29, UL30, UL42, and UL52) comprise the core biochemical activities expected at a replication fork: a processive DNA polymerase, helicase, primase, and a single-stranded DNA-binding protein. Although very little biochemistry has been carried out on the homologs of these genes in other herpesviruses, the available evidence (Ertl and Powell 1992; Tsurumi 1993; Tsurumi et al. 1993a,b,1994) suggests that their function is essentially the same as the HSV proteins. It therefore seems likely, as mentioned earlier, that the characteristic features of herpesvirus lytic DNA replication are due to the conserved enzymological features of this set of six conserved genes. Each herpesvirus also encodes other proteins required for DNA replication. These additional proteins fall into two groups: (1) Transcriptional regulatory proteins required for the expression of the other replication

Table 1 Herpesvirus genes required for lytic DNA replication

HSV[a,b]	EBV[c,d]	HCMV[e,f]	Function
UL30	BALF5	UL54	DNA polymerase
UL42	BMRF1	UL44	polymerase processivity
UL29	BALF2	UL57	single-stranded DNA binding
UL5	BBLF4	UL105	helicase/primase
UL52	BSLF1	UL70	helicase/primase
UL8	BBLF2/3	UL101–102	helicase/primase
UL9			origin binding/helicase
	BZLF1		origin binding/transcription activation
		UL112–113	transcription regulation
		IE1/IE2	transcription regulation
		IRS1	transcription regulation
		UL84	?

The data are summarized from the following sources: [a]Challberg 1986; [b]Wu et al. 1988; [c]Fixman et al. 1992; [d]Fixman et al. 1995; [e]Pari and Anders 1993; [f]Pari et al. 1993.

genes. In the case of HSV, expression of the nonregulatory genes from a constitutive promoter eliminated the requirement for the regulatory genes (Heilbronn and zur Hausen 1989). In the case of EBV, constitutive expression of all of the genes did not eliminate the requirement for the transcriptional activator, BZLF1 (Fixman et al. 1995). This experiment has not yet been completed in the case of HCMV. (2) Origin recognition proteins such as the HSV UL9 protein. In the case of EBV, the requirement for the transcriptional activator protein is due to the fact that BZLF1 also serves as the origin recognition protein (Schepers et al. 1993a). As mentioned earlier, the lack of conservation of origin recognition proteins mirrors the diversity of structure of the origins of different herpesvirus groups.

It should also be noted that the various herpesviruses encode a number of other gene products that play a critical role in DNA replication in the natural host but which are not essential for DNA replication in the rather artificial system of actively dividing immortalized cells in culture. These functions include a number of enzymes involved in nucleotide biosynthesis such as ribonucleotide reductase, thymidine kinase, thymidylate kinase, and thymidylate synthetase. Additionally, it is clear that host proteins play a role in DNA replication as well. As noted, all of the herpesvirus origins contain binding sites for host transcription factors. Moreover, there is evidence in the case of HSV that the virus-encoded origin-binding protein, UL9, interacts specifically with the host DNA polymerase-α (Lee et al. 1995). Even if there are no cellular

proteins that participate directly in the synthetic events at the replication fork, there are other activities that would be predicted to be required for DNA replication that may well be supplied by the host cell. For example, neither a ligase activity nor a topoisomerase activity has been associated with any of the purified viral replication proteins, and there is no report that such activities are induced following infection. One of the major challenges in the future will be to understand how the virus- and host-encoded proteins are integrated into a functional replication system.

ORIGIN BINDING PROTEIN (UL9)

The *ul9* gene encodes a polypeptide of 851 amino acids with a predicted molecular mass of 94 kD. The protein contains at least two functional domains: The amino-terminal two-thirds of the protein mediates dimerization (Elias et al. 1992; Hazuda et al. 1992) as well as a DNA-dependent helicase activity (Bruckner et al. 1991; Fierer and Challberg 1992; Martinez et al. 1992; Boehmer et al. 1993), and the carboxy-terminal domain of 317 amino acids mediates sequence-specific DNA binding (Weir et al. 1989; Hazuda et al. 1991; Arbuckle and Stow 1993; Martinez and Edwards 1993; Perry et al. 1993; Martin et al. 1994; Fierer and Challberg 1995). The amino-terminal two-thirds of UL9 contains six motifs that make it a member of a superfamily of helicases (Gorbalenya et al. 1988, 1989). Genetic evidence has demonstrated that these motifs are essential for viral DNA replication: Mutations in five of the six motifs render the gene unable to support DNA replication in a transient replication system (Martinez et al. 1992). UL9 has been purified to homogeneity both from HSV-infected cells and from insect cells infected with a recombinant baculovirus expressing UL9. The purified protein exhibits DNA-dependent ATPase activity and helicase activity (Bruckner et al. 1991; Fierer and Challberg 1992; Boehmer et al. 1993; Dodson and Lehman 1993). The helicase has a 3′ to 5′ polarity, is able to unwind partially duplex DNA of nonspecific sequence, and does not require a single-stranded tail (Fierer and Challberg 1992; Boehmer et al. 1993). As discussed below, UL9 has not been shown to unwind fully duplex DNA, however, nor to preferentially unwind origin-containing DNA (Fierer and Challberg 1992; Boehmer et al. 1993).

Purified UL9 is a homodimer in solution (Bruckner et al. 1991; Fierer and Challberg 1992). The dimerization domain appears to reside predominantly in the amino-terminal two-thirds of the molecule. Thus, the full-length UL9 is a tightly associated homodimer in solution with an upper limit for the dissociation constant of approximately 1×10^{-10} M. The DNA-binding domain, however, which comprises the carboxy-terminal

317 amino acids (t-UL9), is predominantly a monomer in solution with a dissociation constant of approximately 1×10^{-5} M (D.S. Fierer et al., unpubl.). Several different approaches have been used to determine the number of monomeric UL9 DNA-binding domains that interact with a single binding site, with conflicting results. In one approach, Fab fragment directed at the carboxyl terminus of UL9 was added to a mixture of purified DNA-binding domain and a single UL9-binding site and analyzed by gel mobility shift assay (Stabell and Olivo 1993). Increasing concentrations of the Fab resulted in the appearance of two bands of lower electrophoretic mobility, suggesting that two t-UL9 molecules bind to the site. In another approach (Martin et al. 1994; Gustafsson et al. 1995), two slightly different-sized versions of the UL9 DNA-binding domain were produced. In gel shift experiments in which both of these forms of UL9 were present, only two protein/DNA complexes were observed, the mobilities of which corresponded to the mobilities of the complexes observed with each form of the DNA-binding domain alone. This result suggests that no heterodimers between the larger and smaller polypeptides were formed and, therefore, that the DNA-binding domain binds to a single binding site as a monomer. Finally, the stoichiometry of binding was measured directly by using a double-label gel shift assay (Fierer and Challberg 1995), and the results of this approach showed that UL9 binds to a single site as a dimer. Additional work will be required to reconcile these conflicting results.

There is strong evidence that binding of UL9 to the core origin results in the formation of a complex, higher-order nucleoprotein structure (Elias et al. 1990; Koff et al. 1991; Fierer and Challberg 1992; Gustafsson et al. 1994). The binding of UL9 to sites I and II is cooperative (Elias et al. 1990, 1992; Hazuda et al. 1992), suggesting some form of interaction between the proteins bound at the two sites. DNase I footprinting experiments using derivatives of the origin in which the distance between sites I and II was lengthened have shown that the binding of UL9 results in the formation of a periodic pattern of DNase hypersensitivity in the DNA between the two binding sites, with an interval between hypersensitive sites of 10 bp (Koff et al. 1991; Fierer and Challberg 1992; Stabell and Olivo 1993). This pattern of hypersensitivity is a clear indication that the DNA between the two UL9-binding sites must be held in some sort of fixed conformation, and it has been suggested that protein-protein interaction between UL9 bound at the two sites holds the DNA between the two sites in a loop. In contrast, neither cooperative binding nor the periodic hypersensitivity pattern was observed when the carboxy-terminal UL9-binding domain was used instead

of full-length UL9 (Elias et al. 1990; Stabell and Olivo 1993). Taken together, these results indicate that the amino-terminal two-thirds of UL9 is required for the interaction between UL9 molecules bound to the origin and support the view that protein-protein interactions between UL9 molecules bound at the two sites hold the DNA in a fixed conformation. The current data are consistent with such protein-protein interactions occurring between dimers of UL9 bound at sites I and II, although more complicated models are also possible.

The events following the binding of UL9 to the core origin sequence that lead to the actual initiation of DNA synthesis are less well-understood than the structure of the UL9/DNA complex. Clearly, the two parental strands must be unwound as a prelude to the initiation of daughter-strand synthesis. A reasonable model is that UL9 binds specifically to the origin and unwinds the AT-rich region to allow or direct assembly of the replication machinery. As noted earlier, however, UL9 has not been demonstrated to exhibit origin-unwinding activity, and attempts by several investigators to demonstrate origin-specific unwinding by UL9 have proven unsuccessful (Fierer and Challberg 1992; Boehmer and Lehman 1993). UL9-induced distortions of the DNA in the vicinity of the origin were detected by permanganate footprinting experiments (Koff et al. 1991), but these distortions did not require ATP hydrolysis, as might be expected if they were the result of unwinding catalyzed by the helicase activity of UL9. It seems likely that these distortions reflect the formation of a specific nucleoprotein structure, rather than local unwinding at a site within the origin. Nevertheless, despite the lack of any biochemical confirmation to date, the idea that the helicase activity of UL9 plays an essential role in initiation by unwinding DNA at the origin is still an attractive hypothesis (Martinez et al. 1992). It is possible that the experimental conditions necessary to detect such an unwinding event in vitro have not yet been discovered, or it is possible that there is another component required for the reaction, a cellular protein perhaps, that has not yet been identified. As mentioned, it has been reported that mutations in ori_S that eliminate the binding of the cellular protein, OF-1, diminish the replication efficiency of ori_S in a transient DNA replication assay (Dabrowski and Schaffer 1991; Dabrowski et al. 1994). It has also been reported recently that UL9 interacts with the cellular replication protein DNA polymerase-α (Lee et al. 1995). Future work on UL9 will have to be directed toward understanding the exact role of its helicase activity, the role of the complex nucleoprotein structure that is formed upon binding of the protein to the origins, and the role of cellular proteins that also interact with the origin.

In the model of UL9-initiated HSV DNA replication suggested above, it would be expected that UL9 would interact with the HSV replication proteins present at the newly formed replication fork. Such interactions have been demonstrated between UL9 and at least three other HSV replication proteins. The ssDNA-binding protein, ICP8, has been shown to stimulate the UL9 helicase activity (Fierer and Challberg 1992; Boehmer et al. 1993; Boehmer and Lehman 1993). This stimulation depends on an interaction between ICP8 and the carboxy-terminal DNA-binding domain of UL9. Moreover, deletion of the carboxy-terminal 27 amino acids of UL9 eliminated the interaction between UL9 and ICP8 without affecting the other activities of the protein, and this mutant UL9 did not support DNA replication in an in vivo assay, strongly suggesting that the interaction is critically important for DNA replication (Boehmer et al. 1994). Recently, it has been demonstrated by immunoprecipitation experiments that the helicase/primase complex interacts with UL9 via specific contacts with the UL8 subunit (McLean et al. 1994). The DNA-binding domain of UL9 was found to be dispensable for this interaction; therefore it is possible, at least in principle, for UL9 to interact with both ICP8 and the helicase/primase simultaneously. It has also been reported that UL42, the processivity factor subunit of the DNA polymerase (see below), can be co-immunoprecipitated with UL9 (D. Parris, pers. comm.), and the catalytic subunit of the polymerase has been shown to interact specifically with UL5, the helicase component of the helicase/primase enzyme (J. Crute, pers. comm.). Thus, there is evidence for the interaction of each of the seven essential viral replication proteins with at least one other member of the group. Indeed, it has been reported that the HSV replication proteins can be purified as a large, multimolecular complex when co-expressed from recombinant baculoviruses in insect cells (Skaliter and Lehman 1994). Moreover, the multiple interactions of UL9 with the other replication proteins, in conjunction with the specific DNA-binding activity of UL9, appear to be suited to recruit these seven polypeptides into a large multiprotein "replisome" at an origin of replication.

THE REPLICATION FORK

DNA Polymerase

The HSV polymerase was the first virus-encoded replication protein to be detected and purified, and a great deal of work has gone into the characterization of this enzyme. The polymerase, as isolated from HSV-infected cells, exists exclusively as a heterodimeric complex of UL30 (140 kD), the catalytic subunit (pol), and UL42 (52 kD), a phospho-

protein that binds tightly to double-stranded DNA (Vaughan et al. 1985; Marsden et al. 1987; Gallo et al. 1988; Crute and Lehman 1989; Gottlieb et al. 1990; Hernandez and Lehman 1990). The catalytic subunit contains an intrinsic 3′→5′ exonuclease activity that probably serves a proofreading function to increase the fidelity of DNA synthesis (Powell and Purifoy 1977; Knopf 1979; O'Donnell et al. 1987; Marcy et al. 1990b). It has been reported that the polymerase also contains an intrinsic 5′→3′ exonuclease/RNase H activity, which is presumed to play a role in the removal of primers from the Okazaki fragments made on the lagging strand during semi-discontinuous synthesis (Crute and Lehman 1989). More recently, however, it has been reported that the polymerase can be separated from 5′→3′ exonuclease activity (J. Hall, pers. comm.) and that this activity may be carried out by another virus-encoded polypeptide, the alkaline exonuclease (UL12) (Knopf and Weisshart 1990).

The interaction between the two subunits of the polymerase has been studied in detail. Mutational analysis of UL42 has demonstrated that the amino-terminal two-thirds of the polypeptide is sufficient for interaction with the polymerase as well as for overall function (Digard et al. 1993b; Gao et al. 1993; Hamatake et al. 1993; Tenney et al. 1993a). It has not been possible, however, to identify a single small peptide sequence or subdomain within the amino-terminal functional domain that is both necessary and sufficient for interaction with pol (Monahan et al. 1993). In contrast, mutational analysis of the pol polypeptide has revealed a short peptide sequence located near the carboxyl terminus of the protein that is sufficient for the interaction of pol with UL42 (Digard et al. 1993a; Tenney et al. 1993b). Deletion of this sequence eliminated the ability of pol to support DNA replication in vivo, despite the fact that the deletion was shown to have no effect on the activity of the enzyme in standard in vitro assays (Digard et al. 1993a; Stow 1993; Tenney et al. 1993b). It seems likely, therefore, that the interaction of pol and UL42 is critical for DNA replication.

The viral pol/UL42 polymerase holoenzyme is a highly processive enzyme, able to synthesize long strands without dissociating from the template strand, and it is the UL42 subunit of the polymerase that is responsible for the high processivity of the enzyme (Gottlieb et al. 1990; Hernandez and Lehman 1990). The mechanism of this increased processivity has been examined using a model primer template composed of a hairpin duplex region with a 5′ single-stranded sequence (Gottlieb and Challberg 1994). Nuclease protection experiments showed that pol bound this substrate at the single-stranded/double-stranded junction (mimicking the primer-template junction), whereas pol/UL42 had a foot-

print that extended further into the downstream duplex region of the substrate. In contrast to pol alone, UL42 alone did not interact specifically with a primer-template junction but rather interacted nonspecifically with the double-stranded portion of the substrate and did not interact to a detectable degree with the single-stranded region. These findings are consistent with previous studies on the DNA-binding properties of UL42, which showed that this protein is a double-stranded-specific DNA-binding protein (Vaughan et al. 1985; Gallo et al. 1988) In addition, the pol/UL42 complex had an overall 10-fold higher affinity for the substrate than pol alone. Taken together, these data suggest that the double-stranded DNA-binding property of the UL42 subunit acts to increase the processivity of the polymerase by forming a "sliding clamp," anchoring the polymerase to the newly synthesized duplex DNA and decreasing the probability that the polymerase dissociates from the template strand after each round of catalysis. Mutational analysis of the two polypeptides also supports this model. First, there is a tight correlation between processivity and the ability of the two proteins to interact (Digard and Coen 1990; Digard et al. 1993a,b; Gao et al. 1993; Hamatake et al. 1993; Monahan et al. 1993; Tenney et al. 1993a,b). Second, mutants of UL42 that lack double-stranded DNA-binding activity but still retain the ability to interact with pol also do not support processive synthesis (Chow and Coen 1995).

The available data thus suggest that UL42 and its homologs in other herpesviruses represent a novel class of polymerase processivity factors whose mechanism is quite different from some of the better-characterized proteins such as the β subunit of *Escherichia coli* Pol III, the eukaryotic replication factor PCNA, and the gene 45 protein for T4 phage DNA polymerase (for review, see Kuriyan and O'Donnell 1993). The mechanism by which UL42 acts differs from these other proteins in a number of ways. First, UL42 has high intrinsic affinity for DNA. Second, it forms a tight heterodimeric complex with the DNA polymerase, and there is no evidence that UL42 is assembled into a multimeric torus around DNA. Finally, the function of UL42 does not require the participation of a primer recognition protein nor does it require ATP. UL42 appears to be most similar to the thioredoxin subunit of the T7 DNA polymerase, which is thought to function by causing a decrease in the dissociation rate of the polymerase from the primer template (Tabor et al. 1987; Marians 1992). A key question that now remains to be answered concerns the mechanistic basis for sliding along DNA by proteins like UL42 that have a high intrinsic affinity for DNA. It seems likely that the answer to this question will require detailed structural information.

Single-stranded DNA-binding Protein ICP8

Infected-cell protein 8 (ICP8; product of UL29 gene) was recognized many years ago as an abundant HSV-induced protein of about 130 kD that binds tightly to single-stranded DNA cellulose columns (Honess and Roizman 1973; Bayliss et al. 1975; Powell and Courtney 1975; Powell and Purifoy 1976; Powell et al. 1981). ICP8 has many of the properties that are characteristic of helix-destabilizing proteins. It binds more tightly to single-stranded DNA than to double-stranded DNA (Ruyechan and Weir 1984), and its binding to single-stranded DNA is cooperative and independent of sequence (Ruyechan 1983; Ruyechan and Weir 1984). Purified ICP8 destabilizes short duplex DNA segments and promotes the renaturation of long complementary strands (Bortner et al. 1993; Dutch and Lehman 1993). It seems reasonable to assume that the function of ICP8 is analogous to that of other single-stranded DNA-binding proteins: to bind to the single-stranded DNA formed at a replication fork by the unwinding of the parental duplex DNA and to facilitate the use of these strands as templates for DNA polymerase. It has been reported that purified ICP8 has a small (no greater than 2-fold) stimulatory effect on the activity of purified HSV DNA polymerase using activated DNA as template (Ruyechan and Weir 1984), and using a single-stranded M13 DNA template, ICP8 was shown to be required for the processive synthesis of long DNA strands (Hernandez and Lehman 1990).

There is also evidence suggesting that ICP8 may interact specifically with other replication proteins. As noted earlier, ICP8, but not heterologous SSBs (Fierer and Challberg 1992; Boehmer and Lehman 1993), stimulates the helicase activity of UL9 on substrates having long duplex regions. Several *ts* mutants with defects in ICP8 have been shown to display altered sensitivities (at the permissive temperature) to drugs that inhibit the viral DNA polymerase (Chiou et al. 1985). ICP8 stimulates both the helicase and primase activities of the HSV helicase/primase (R. Hamatake and D. Tenney, pers. comm., D. Klinedinst and M. Challberg, unpubl.). Finally, protein affinity chromatography experiments have suggested that ICP8 interacts with UL42 and with the virus-encoded nuclease, UL12 (Vaughan et al. 1984; Thomas et al. 1992). The functional significance of the interaction between ICP8 and the UL12 nuclease is not clear, although it is worth noting that the nuclease appears to be necessary for the processing of branched replication intermediates (Shao et al. 1993) and may be responsible for removing the RNA primers at the 5′ end of Okazaki fragments (Knopf and Weisshart 1990). It seems likely, therefore, that ICP8 plays a role in organizing a multiprotein complex at the replication fork by providing specific contacts

with the other replication proteins. It is also possible that at least some of these observations reflect a role for ICP8 in organizing or maintaining the large subnuclear structures, known as replication compartments, in which viral DNA replication takes place (Quinlan et al. 1984; de Bruyn Kops and Knipe 1988). More detailed molecular genetic analyses of ICP8, as well as the continued development and analysis of in vitro reactions involving combinations of the viral replication proteins, should help to clarify some of these issues.

Helicase/Primase UL5/UL8/UL52

Infection of cells with HSV induces novel helicase and primase activities (Crute et al. 1988). These two activities have been purified to homogeneity; both helicase and primase activities are components of a three-subunit enzyme comprising the products of the *ul5, ul8,* and *ul52* genes (Crute et al. 1989; Dodson et al. 1989; Crute and Lehman 1991).

The helicase activity of this enzyme has been extensively characterized. The helicase can utilize either ATP or GTP as a co-factor for unwinding (Crute et al. 1988; Crute and Lehman 1991). The activity of the helicase on model substrates suggests that it moves in the 5′ to 3′ direction on the strand to which it is bound and requires a single-stranded tail on the fragment which is being displaced (Crute et al. 1988). The predicted UL5 amino acid sequence contains the six sequence motifs that are characteristic of helicases (Gorbalenya et al. 1988, 1989; Hodgman 1988; Lane 1988; McGeoch et al. 1988), and there is genetic evidence that mutations in highly conserved residues within each of these six motifs render the protein inactive in DNA synthesis (Zhu and Weller 1992b). Even though UL5 can be purified in soluble form, the isolated UL5 polypeptide has only weak, if any, activity, as a helicase in the absence of UL52. Co-expression of UL5 and UL52, however, results in the formation of a heterodimeric complex that has full helicase activity on standard substrates (Calder and Stow 1990; Dodson and Lehman 1991; Sherman et al. 1992). The UL8 subunit, therefore, is not required for helicase activity per se, although as indicated below, UL8 may affect helicase activity in more complex assays.

The primase activity of this enzyme has also been extensively characterized. It was recently shown that the UL52 polypeptide is responsible for the primase activity of the helicase/primase complex (Klinedinst and Challberg 1994; Dracheva et al. 1995). A sequence motif in UL52 that is highly conserved in the UL52 homologs of other herpesviruses contains a DXD element that is similar to the divalent metal-binding site of DNA polymerases and several DNA primases. Mutation of either of the

aspartic acid residues in this motif abolished the ability of the protein to support replication in vivo, and completely eliminated primase activity in vitro, with no effect on the ATPase and helicase activities of the enzyme. It has not been possible to obtain sufficient quantities of soluble UL52 to determine whether the isolated protein has enzymatic activity in the absence of UL5, although the heterodimeric UL5/UL52 complex does have detectable primase activity (Dodson and Lehman 1991; Sherman et al. 1992; Tenney et al. 1994). Elimination of helicase activity by specific mutation of the nucleoside triphosphate binding site in UL5 had no effect on primase activity (J. Crute and J. Gottlieb, pers. comm.). The predominant products of the primase are oligoribonucleotides 8–10 residues in length (Crute and Lehman 1991; Sherman et al. 1992; Tenney et al. 1994, 1995). Unlike the primase activity of the eukaryotic pol-α:primase enzyme, the HSV primase has a strong preference for certain template sequences (Tenney et al. 1995; J. Gottlieb and M. Challberg, unpubl.). The current understanding of the rules for template recognition and primer synthesis are as follows: (1) The minimal required sequence consists of a deoxyguanosine followed (3′–5′) by a stretch of at least five pyrimidines. The five pyrimidines can consist entirely of deoxycytidines, but not entirely of thymidines. (2) The deoxyguanosine residue at the 3′ end of this sequence is absolutely required for primase recognition, but it is not a template for the first nucleotide of the primer; primer synthesis begins at the second nucleotide in the template sequence. (3) Deoxyadenosine or deoxyguanosine substitutions are tolerated after the second pyrimidine (GYY...), although they do reduce overall activity. (4) Primer synthesis terminates after the incorporation of 7–15 nucleotides, regardless of sequence; purines in the template increase the probability that primer synthesis will terminate prematurely.

The function of UL8 is currently not well understood. As mentioned earlier, although UL8 is not absolutely required for primase activity, the absence of UL8 does reduce primer synthesis about 10-fold on long single-stranded DNA templates (Tenney et al. 1994); UL8 does not, however, appear to have an effect on the template requirements of the enzyme or on the nature of the product oligoribonucleotides. Recent findings suggest that UL8 has an additional activity that is distinguishable biochemically from its effect on the primase (J. Gottlieb and M. Challberg, unpubl.). DNA synthesis was reconstituted with purified HSV fork proteins and a preformed replication fork substrate consisting of a single-stranded DNA circle primed with an oligonucleotide containing an unpaired 5′ single-stranded tail. In this system, efficient leading-strand synthesis, in which the product DNA strands ranged in size from 1 to

more than 10 times the unit length of the substrate, required the HSV DNA polymerase, the single-stranded DNA-binding protein ICP8, and all three subunits of the UL5/UL8/UL52 helicase/primase; reactions lacking UL8 failed to carry out displacement synthesis. The mechanism of these two effects of UL8 is not known. It is possible that the two activities reflect two distinct functions of the UL8 polypeptide, or that both activities reflect the same underlying function. One possibility, for example, is that UL8 increases the processivity of the helicase/primase. If so, then the mechanism of increased processivity is more complex than simply to act as a clamp as UL42 does for the DNA polymerase, since although UL8 obviously interacts with the catalytic subunits of the enzyme, UL8 alone does not bind appreciably to single-stranded DNA (Parry et al. 1993).

CONCLUDING REMARKS

The biochemical functions of the herpesvirus-encoded replication proteins are now reasonably well understood, although clearly there are many outstanding questions. It seems likely that continued enzymological and molecular genetic studies will provide many answers to these questions in the future. Two key questions concern the mechanism of initiation and the potential involvement of cellular proteins in the lytic replication process. As indicated, some progress has been made in these areas, but a major stumbling block to additional progress continues to be the lack of good in vitro systems that clearly mimic the events that occur in vivo. There are now several reports of in vitro systems that carry out origin-independent rolling-circle-type replication that is dependent on the virus-encoded fork proteins (Rabkin and Hanlon 1990; Skaliter and Lehman 1994). Continued analysis of these systems should shed additional light on the ways in which the viral proteins interact at a replication fork to increase the overall efficiency of the individual components and may provide insight into the role of cellular proteins in the events that follow initiation. What is still missing is an in vitro system in which origin-dependent initiation takes place, and a complete understanding of herpesvirus replication will depend on the development of such a system.

REFERENCES

Anders, D.G. and S.M. Punturieri. 1991. Multicomponent origin of cytomegalovirus lytic-phase DNA replication. *J. Virol.* **65:** 931–937.

Anders, D.G., M.A. Kacica, G. Pari, and S.M. Punturieri. 1992. Boundaries and structure

of human cytomegalovirus oriLyt, a complex origin for lytic-phase DNA replication. *J. Virol.* **66:** 3373–3384.

Arbuckle, M.I. and N.D. Stow. 1993. A mutational analysis of the DNA-binding domain of the herpes simplex virus type 1 UL9 protein. *J. Gen. Virol.* **74:** 1349–1355.

Bataille, D. and A. Epstein. 1994. Herpes simplex virus replicative concatemers contain L components in inverted orientation. *Virology* **203:** 384–388.

Bayliss, G.J., H.S. Marsden, and J. Hay. 1975. Herpes simplex virus proteins: DNA binding proteins in infected cells and in the virus structure. *Virology* **68:** 124–134.

Boehmer, P.E. and I.R. Lehman. 1993. Physical interaction between the herpes simplex virus 1 origin-binding protein and single-stranded DNA-binding protein ICP8. *Proc. Natl. Acad. Sci.* **90:** 8444–8448.

Boehmer, P.E., M.S. Dodson, and I.R. Lehman. 1993. The herpes simplex virus type I origin binding protein: DNA helicase activity. *J. Biol. Chem.* **268:** 1220–1225.

Boehmer, P., M. Craigie, N. Stow, and I.R. Lehman. 1994. Association of origin binding protein and single strand DNA-binding protein, ICP8, during herpes simplex virus type 1 DNA replication in vivo. *J. Biol. Chem.* **269:** 29329–29334.

Bortner, C., T.R. Hernandez, I.R. Lehman, and J. Griffith. 1993. Herpes simplex virus 1 single-stranded DNA-binding protein (ICP8) will promote homologous pairing and strand transfer. *J. Mol. Biol.* **231:** 241–250.

Bruckner, R.C., J.J. Crute, M.S. Dodson, and I.R. Lehman. 1991. The herpes simplex virus 1 origin binding protein: A DNA helicase. *J. Biol. Chem.* **266:** 2669–2674.

Calder, J.M. and N.D. Stow. 1990. Herpes simplex virus helicase-primase: The UL8 protein is not required for DNA-dependent ATPase and DNA helicase activities. *Nucleic Acids Res.* **25:** 3573–3578.

Carmichael, E.P. and S.K. Weller. 1989. Herpes simplex virus type 1 DNA synthesis requires the product of the UL8 gene: Isolation and characterization of an ICP6:lacZ insertion mutation. *J. Virol.* **63:** 591–599.

Carmichael, E.P., M. Kosovosky, and S.K. Weller. 1988. Isolation and characterization of herpes simplex virus type 1 host range mutants defective in viral DNA synthesis. *J. Virol.* **62:** 91–99.

Challberg, M.D. 1986. A method for identifying the viral genes required for herpesvirus DNA replication. *Proc. Natl. Acad. Sci.* **83:** 9094–9098.

Chartrand, P., C.S. Crumpacker, P.A. Schaffer, and N.M. Wilkie. 1980. Physical and genetic analysis of the herpes simplex virus DNA polymerase locus. *Virology* **103:** 311–326.

Cheng, L. and T.J. Kelly. 1989. Transcriptional activator nuclear factor I stimulates the replication of SV40 minichromosomes in vivo and in vitro. *Cell* **59:** 541–551.

Chiou, H.C., S.K. Weller, and D.M. Coen. 1985. Mutations in the herpes simplex virus major DNA-binding protein gene leading to altered sensitivity to DNA polymerase inhibitors. *Virology* **145:** 213–226.

Chow, C.S. and D.M. Coen. 1995. Mutations that specifically impair DNA binding activity of the herpes simplex virus protein UL42. *J. Viol.* **69:** 6965–6971.

Coen, D.M., D.P. Aschman, P.T. Gelep, M.J. Retondo, S.K. Weller, and P.A. Schaffer. 1984. Fine mapping and molecular cloning of mutations in the herpes simplex virus DNA polymerase locus. *J. Virol.* **49:** 236–247.

Conley, A.J., D.M. Knipe, P. Jones, and B. Roizman. 1981. Molecular genetics of herpes simplex virus. VII. Characterization of a temperature-sensitive mutant produced by in vitro mutagenesis and defective in DNA synthesis and accumulation of gamma

polypeptides. *J. Virol.* **37:** 191–206.

Crute, J.J. and I.R. Lehman. 1989. Herpes simplex-1 DNA polymerase. Identification of an intrinsic 5′ to 3′ exonuclease with ribonuclease H activity. *J. Biol. Chem.* **264:** 19266–19270.

———. 1991. Herpes simplex virus-1 helicase-primase. Physical and catalytic properties. *J. Biol. Chem.* **266:** 4484–4488.

Crute, J.J., E.S. Mocarski, and I.R. Lehman. 1988. A DNA helicase induced by herpes simplex virus type 1. *Nucleic Acids Res.* **16:** 6585–6596.

Crute, J.J., T. Tsurumi, L. Zhu, S.K. Weller, P.D. Olivo, M.D. Challberg, E.S. Mocarski, and I.R. Lehman. 1989. Herpes simplex virus 1 helicase-primase: A complex of three herpes-encoded gene products. *Proc. Natl. Acad. Sci.* **86:** 2186–2189.

Dabrowski, C.E. and P.A. Schaffer. 1991. Herpes simplex virus type 1 origin-specific binding protein: oriS-binding properties and effects of cellular proteins. *J. Virol.* **65:** 3140–3150.

Dabrowski, C.E., P.J. Carmillo, and P.A. Schaffer. 1994. Cellular protein interactions with herpes simplex virus type 1 oriS. *Mol. Cell. Biol.* **14:** 2545–2555.

de Bruyn Kops, A. and D.M. Knipe. 1988. Formation of DNA replication structures in herpes virus-infected cells requires a viral DNA binding protein. *Cell* **55:** 857–868.

Deb, S. and S.P. Deb. 1989. Analysis of Ori-S sequence of HSV-1: Identification of one functional DNA binding domain. *Nucleic Acids Res.* **17:** 2733–2752.

Digard, P. and D.M. Coen. 1990. The binding site on the herpes simplex virus polymerase for the viral UL42 protein. *J. Biol. Chem.* **265:** 17393–17396.

Digard, P., W.R. Bebrin, K. Weisshart, and D.M. Coen. 1993a. The extreme C terminus of herpes simplex virus DNA polymerase is crucial for functional interaction with processivity factor UL42 and for viral replication. *J. Virol.* **67:** 398–406.

Digard, P., C.S. Chow, L. Pirrit, and D.M. Coen. 1993b. Functional analysis of the herpes simplex virus UL42 protein. *J. Virol.* **67:** 1159–1168.

Dodson, M.S. and I.R. Lehman. 1991. Association of DNA helicase and primase activities with a subassembly of the herpes simplex virus 1 helicase-primase composed of the UL5 and UL52 gene products. *Proc. Natl. Acad. Sci.* **88:** 1105–1109.

———. 1993. The herpes simplex virus type I origin binding protein. *J. Biol. Chem.*. **268:** 1213–1219.

Dodson, M.S., J.J. Crute, R.C. Bruckner, and I.R. Lehman. 1989. Overexpression and assembly of the herpes simplex virus type-1 helicase-primase in insect cells. *J. Biol. Chem.* **264:** 20835–20838.

Dracheva, S., E. Koonin, and J.J. Crute. 1995. Identification of the primase active site of the herpes simplex virus type 1 helicase–primase. *J. Biol. Chem.* **270:** 14148–14153.

Dutch, R.E. and I.R. Lehman. 1993. Renaturation of complementary DNA strands by herpes simplex virus type 1 ICP8. *J. Virol.* **67:** 6945–6949.

Dutch, R.E., V. Bianchi, and I.R. Lehman. 1995. Herpes simplex virus type 1 DNA replication is specifically required for high-frequency homologous recombination between repeated sequences. *J. Virol.* **69:** 3084–3089.

Dutch, R.E., R.C. Bruckner, E.S. Mocarski, and I.R. Lehman. 1992. Herpes simplex virus type 1 recombination: Role of DNA replication and viral a sequences. *J. Virol.* **66:** 277–285.

Elias, P. and I.R. Lehman. 1988. Interaction of origin binding protein with an origin of replication of herpes simplex virus 1. *Proc. Natl. Acad. Sci.* **85:** 2959–2963.

Elias, P., C.M. Gustafsson, and O. Hammarsten. 1990. The origin binding protein of

herpes simplex virus 1 binds cooperatively to the viral origin of replication. *J. Biol. Chem.* **265:** 17167–17173.

Elias, P., C.M. Gustafsson, O. Hammarsten, and N.D. Stow. 1992. Structural elements required for the cooperative binding of the herpes simplex virus origin binding protein to oriS reside in the N-terminal part of the protein. *J. Biol. Chem.* **267:** 17424–17429.

Ertl, P.F. and K.L. Powell. 1992. Physical and functional interaction of human cytomegalovirus DNA polymerase and its accessory protein (ICP36) expressed in insect cells. *J. Virol.* **66:** 4126–4133.

Fierer, D.S. and M.D. Challberg. 1992. Purification and characterization of UL9, the herpes simplex virus type 1 origin-binding protein. *J. Virol.* **66:** 3986–3995.

———. 1995. The stoichiometry of binding of the herpes simplex virus type 1 origin binding protein, UL9, to oriS. *J. Biol. Chem.* **270:** 7330–7334.

Fixman, E.D., G.S. Hayward, and S.D. Hayward. 1992. *trans*-Acting requirements for replication of Epstein-Barr virus ori-Lyt. *J. Virol.* **66:** 5030–5039.

———. 1995. Replication of Epstein-Barr virus oriLyt: Lack of a dedicated virally encoded origin-binding protein and dependence on Zta in cotransfection assays. *J. Virol.* **69:** 2998–3006.

Frenkel, N., H. Locker, W. Batterson, G.S. Hayward, and B. Roizman. 1976. Anatomy of herpes simplex virus DNA. VI. Defective DNA originates from the S component. *J. Virol.* **20:** 527–531.

Frenkel, N., R.J. Jacob, R.W. Honess, G.S. Hayward, H. Locker, and B. Roizman. 1975. Anatomy of herpes simplex virus DNA. III. Characterization of defective DNA molecules and biological properties of virus populations containing them. *J. Virol.* **16:** 153–167.

Gallo, M.L., D.H. Jackwood, M. Murphy, H.S. Marsden, and D.S. Parris. 1988. Purification of the herpes simplex virus type 1 65-kilodalton DNA-binding protein: Properties of the protein and evidence of its association with the virus-encoded DNA polymerase. *J. Virol.* **62:** 2874–2883.

Gao, M., S.F. DiTusa, and M.G. Cordingley. 1993. The C-terminal third of UL42, a HSV-1 DNA replication protein, is dispensable for viral growth. *Virology* **194:** 647–653.

Garber, D.A., S.M. Beverley, and D.M. Coen. 1993. Demonstration of circularization of herpes simplex virus DNA following infection using pulsed field gel electrophoresis. *Virology* **197:** 459–462.

Goldstein, D.J. and S.K. Weller. 1988. An ICP6::lacZ insertion mutagen is used to demonstrate that the *UL52* gene of herpes simplex virus type 1 is required for virus growth and DNA synthesis. *J. Virol.* **62:** 2970–2977.

Gorbalenya, A.E., E.V. Koonin, A.P. Donchenko, and V.M. Blinov. 1988. A conserved NTP-motif in putative helicases. *Nature* **333:** 22.

———. 1989. Two related superfamilies of putative helicases involved in replication, repair and expression of DNA and RNA genomes. *Nucleic Acids Res.* **17:** 4713–4730.

Gottlieb, J. and M.D. Challberg. 1994. Interaction of herpes simplex virus type 1 DNA polymerase and the UL42 accessory protein with a model primer template. *J. Virol.* **68:** 4937–4945.

Gottlieb, J., A.I. Marcy, D.M. Coen, and M.D. Challberg. 1990. The herpes simplex virus type 1 *UL42* gene product: A subunit of DNA polymerase that functions to increase processivity. *J. Virol.* **64:** 5976–5987.

Gustafsson, C.M., O. Hammarsten, M. Falkenberg, and P. Elias. 1994. Herpes simplex

virus DNA replication: A spacer sequence directs the ATP-dependent formation of a nucleoprotein complex at oriS. *Proc. Natl. Acad. Sci.* **91:** 4629–4633.

Gustafsson, C.M., M. Falkenberg, S. Simonsson, H. Valadi, and P. Elias. 1995. The DNA ligands influence the interactions between the herpes simplex virus 1 origin binding protein and the single strand DNA-binding protein, ICP-8. *J. Biol. Chem.* **270:** 19028–19034.

Hamatake, R.K., M. Bifano, D.J. Tenney, W.W. Hurlburt, and M.G. Cordingley. 1993. The herpes simpex virus type 1 DNA polymerase accessory protein, UL42, contains a functional protease-resistant domain. *J. Gen. Virol.* **74:** 2181–2189.

Hamzeh, F.M., P.S. Lietman, W. Gibson, and G.S. Hayward. 1990. Identification of the lytic origin of DNA replication in human cytomegalovirus by a novel approach utilizing ganciclovir-induced chain termination. *J. Virol.* **64:** 6184–6195.

Hardwicke, M.A. and P.A. Schaffer. 1995. Cloning and characterization of herpes simplex virus type 1 oriL: Comparison of replication and protein-DNA complex formation by oriL and oriS. *J. Virol.* **69:** 1377–1388.

Hazuda, D.J., H.C. Perry, and W.L. McClements. 1992. Cooperative interactions between replication origin-bound molecules of herpes simplex virus origin-binding protein are mediated via the aminoterminus of the protein. *J. Biol. Chem.* **267:** 14309–14315.

Hazuda, D.J., H.C. Perry, A.M. Naylor, and W.L. McClements. 1991. Characterization of the herpes simplex virus origin binding protein interaction with oriS. *J. Biol. Chem.* **266:** 24621–24626.

Heilbronn, R. and H. zur Hausen. 1989. A subset of herpes simplex virus replication genes induces DNA amplification within the host cell genome. *J. Virol.* **63:** 3683–3692.

Hernandez, T.R. and I.R. Lehman. 1990. Functional interaction between the herpes simplex-1 DNA polymerase and UL42 protein. *J. Biol. Chem.* **265:** 11227–11232.

Hernandez, T.R., R.E. Dutch, I.R. Lehman, C. Gustafsson, and P. Elias. 1991. Mutations in a herpes simplex virus type 1 origin that inhibit replication. *J. Virol.* **65:** 1649–1652.

Hodgman, T.C. 1988. A new superfamily of replicative proteins. *Nature* **333:** 22–23.

Honess, R.W. and B. Roizman. 1973. Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstructural herpes virus polypeptides in the infected cell. *J. Virol.* **12:** 1346–1365.

Honess, R.W., A. Buchan, I.W. Halliburton, and D.H. Watson. 1980. Recombination and linkage between structural and regulatory genes of herpes simplex virus type 1: Study of the functional organization of the genome. *J. Virol.* **34:** 716–742.

Hubenthal-Vass, J., L. Starr, and B. Roizman. 1987. The herpes simplex virus origins of DNA synthesis in the S component are each contained in a transcribed open reading frame. *J. Virol.* **61:** 3349–3355.

Igarashi, K., R. Fawl, R.J. Roller, and B. Roizman. 1993. Construction and properties of a recombinant herpes simplex virus 1 lacking both S-component origins of DNA synthesis. *J. Virol.* **67:** 2123–2132.

Jacob, R. and B. Roizman. 1977. Anatomy of herpes simplex virus DNA. VIII. Properties of the replicating DNA. *J. Virol.* **23:** 394–411.

Jacob, R.J., L.S. Morse, and B. Roizman. 1979. Anatomy of herpes simplex virus DNA. XII. Accumulation of head-to-tail concatemers in nuclei of infected cells and their role in the generation of the four isomeric arrangements of viral DNA. *J. Virol.* **29:** 448–457.

Johnson, P.A., M.G. Best, T. Friedman, and D.S. Parris. 1991. Isolation of a herpes

simplex virus type 1 mutant deleted for the essential *UL42* gene and characterization of its null phenotype. *J. Virol.* **65:** 700–710.

Jongeneel, C.V. and S.L. Bachenheimer. 1981. Structure of replicating herpes simplex virus DNA. *J. Virol.* **39:** 656–660.

Klinedinst, D.K. and M.D. Challberg. 1994. Helicase-primase complex of herpes simplex virus type 1: A mutation in the UL52 subunit abolishes primase activity. *J. Virol.* **68:** 3693–3701.

Knopf, C.W. and K. Weisshart. 1990. Comparison of exonucleolytic activities of herpes simplex virus type-1 DNA polymerase and DNase. *Eur. J. Biochem.* **191:** 263–273.

Knopf, C.W., B. Spies, and H.C. Kaerner. 1986. The DNA replication origins of herpes simplex virus type 1 strain Angelotti. *Nucleic Acids Res.* **14:** 8655–8667.

Knopf, K.W. 1979. Properties of herpes simplex virus DNA polymerase and characterization of its associated exonuclease activity. *Eur. J. Biochem.* **98:** 231–244.

Koff, A. and P. Tegtmeyer. 1988. Characterization of major recognition sequences for a herpes simplex virus type 1 origin-binding protein. *J. Virol.* **62:** 4096–4103.

Koff, A., J.F. Schwedes, and P. Tegtmeyer. 1991. Herpes simplex virus origin-binding protein (UL9) loops and distorts the viral replication origin. *J. Virol.* **65:** 3284–3292.

Kuriyan, J. and M. O'Donnell. 1993. Sliding clamps of DNA polymerases. *J. Mol. Biol.* **234:** 915–925.

Lane, D. 1988. Enlarged family of putative helicases. *Nature* **334:** 478.

Lee, S.S.-K., Q. Dong, T.S.-F. Wang, and I.R. Lehman. 1995. Interaction of herpes simplex virus 1 origin-binding protein with DNA polymerase α. *Proc. Natl. Acad. Sci.* **92:** 7882–7886.

Leinbach, S.S. and W.C. Summers. 1980. The structure of herpes simplex virus type 1 DNA as probed by micrococcal nuclease digestion. *J. Gen. Virol.* **51:** 45–59.

Lockshon, D. and D.A. Galloway. 1988. Sequence and structural requirements of a herpes simplex viral DNA replication origin. *Mol. Cell. Biol.* **8:** 4018–4027.

Marchetti, M.E., C.A. Smith, and P.A. Schaffer. 1988. A temperature-sensitive mutation in an HSV-1 gene required for viral DNA synthesis maps to coordinates 0.609-0.614 in UL. *J. Virol.* **62:** 715–721.

Marcy, A.I., C.B.C. Hwang, K.L. Ruffner, and D.M. Coen. 1990a. Engineered herpes simplex virus DNA polymerase point mutants: The most highly conserved region shared among alpha-like DNA polmerases is involved in substrate recognition. *J. Virol.* **64:** 5883–5890.

Marcy, A.I., P.D. Olivo, M.D. Challberg, and D.M. Coen. 1990b. Enzymatic activities of overexpressed herpes simplex virus DNA polymerase purified from recombinant baculovirus-infected insect cells. *Nucleic Acids Res.* **18:** 1207–1215.

Marians, K.J. 1992. Prokaryotic DNA replication. *Annu. Rev. Biochem.* **61:** 673–719.

Marsden, H.S., E.M. Cambell, L. Haarr, M.C. Frame, D.S. Parris, M. Murphy, R.G. Hope, M.T. Muller, and C.M. Preston. 1987. The 65,000 M_r DNA-binding and virion trans-inducing proteins of herpes simplex virus type 1. *J. Virol.* **61:** 2428–2437.

Martin, D.W., R.M. Muñoz, D. Oliver, M.A. Subler, and S. Deb. 1994. Analysis of the DNA-binding domain of the HSV-1 origin-binding protein. *Virology* **198:** 71–80.

Martinez, R. and C.A. Edwards. 1993. Expression, purification, and functional characterization of the DNA-binding domain of the herpes simplex virus type 1 UL9 protein. *Protein Expr. Purif.* **4:** 32–37.

Martinez, R., L. Shao, and S.K. Weller. 1992. The conserved helicase motifs of the herpes simplex virus type 1 origin-binding protein UL9 are important for function. *J.*

Virol. **66:** 6735–6746.

Masse, M.J., S. Karlin, G.A. Schachtel, and E.S. Mocarski. 1992. Human cytomegalovirus origin of DNA replication (oriLyt) resides within a highly complex repetitive region. *Proc. Natl. Acad. Sci.* **89:** 5246–5250.

McGeoch, D.J., M.A. Dalrymple, A. Dolan, D. McNab, L.J. Perry, P. Taylor, and M.D. Challberg. 1988. Structures of herpes simplex virus type 1 genes required for replication of virus DNA. *J. Virol.* **62:** 444–453.

McLean, G.W., A.P. Abbotts, M.E. Parry, H.S. Marsden, and N.D. Stow. 1994. The herpes simplex virus type 1 origin-binding protein interacts specifically with the viral UL8 protein. *J. Gen. Virol.* **75:** 2699–2706.

McVoy, M.A. and S.P. Adler. 1994. Human cytomegalovirus DNA replicates after early circularization by concatemer formation, and inversion occurs within the concatemer. *J. Virol.* **68:** 1040–1051.

Mocarski, E.S. and B. Roizman. 1982. Structure and role of the herpes simplex virus DNA termini in inversion, circularization and generation of virion DNA. *Cell* **31:** 89–97.

Monahan, S.J., T.F. Barlam, C.S. Crumpacker, and D.S. Parris. 1993. Two regions of the herpes simplex virus type 1 UL42 protein are required for its functional interaction with the viral DNA polymerase. *J. Virol.* **67:** 5922–5931.

Murchie, M.J. and D.J. McGeoch. 1982. DNA sequence analysis of an immediate-early gene region of the herpes simplex virus type 1 genome (map coordinates 0.950 to 0.978). *J. Gen. Virol.* **62:** 1–15.

O'Donnell, M.E., P. Elias, and I.R. Lehman. 1987. Processive replication of single-stranded DNA templates by the herpes simplex virus-induced DNA polymerase. *J. Biol. Chem.* **262:** 4252–4259.

Olivo, P.D., N.J. Nelson, and M.D. Challberg. 1988. Herpes simplex virus DNA replication: The UL9 gene encodes an origin binding protein. *Proc. Natl. Acad. Sci.* **85:** 5414–5418.

Orberg, P.K. and P.A. Schaffer. 1987. Expression of herpes simplex virus type 1 major DNA-binding protein, ICP8, in transformed cell lines: Complementation of deletion mutants and inhibition of wild-type virus. *J. Virol.* **61:** 1136–1146.

Pari, G.S. and D.G. Anders. 1993. Eleven loci encoding *trans*-acting factors are required for transient complementation of human cytomegalovirus oriLyt-dependent DNA replication. *J. Virol.* **67:** 6979–6988.

Pari, G.S., M.A. Kacica, and S.D.G. Ander. 1993. Open reading frames UL44, IRS1/TRS1, and UL36-38 are required for transient complementation of human cytomegalovirus oriLyt-dependent DNA synthesis. *J. Virol.* **67:** 2575–2582.

Parry, M.E., N.D. Stow, and H.S. Marsden. 1993. Purification and properties of the herpes simplex virus type 1 UL8 protein. *J. Gen. Virol.* **74:** 607–612.

Perry, H.C., D.J. Hazuda, and W.L. McClements. 1993. The DNA binding domain of herpes simplex virus type 1 origin binding protein is a transdominant inhibitor of virus replication. *Virology* **193:** 73–79.

Poffenberger, K.L. and B. Roizman. 1985. A noninverting genome of a viable herpes simplex virus 1: Presence of head-to-tail linkages in packaged genomes and requirements for circularization after infection. *J. Virol.* **53:** 587–595.

Polvino-Bodnar, M., P.D. Orberg, and P.A. Schaffer. 1987. Herpes simplex virus type 1 oriL is not required for virus replication or for the establishment and reactivation of latent infection in mice. *J. Virol.* **61:** 3528–3535.

Powell, K.L. and R.J. Courtney. 1975. Polypeptides synthesized in herpes simplex virus type 2-infected Hep-2 cells. *Virology* **66:** 217–228.

Powell, K.L. and D.J.M. Purifoy. 1976. DNA-binding proteins induced by herpes simplex virus type 2 in Hep-2 cells. *J. Virol.* **19:** 717–731.

———. 1977a. Nonstructural proteins of herpes simplex virus. I. Purification of the induced DNA polymerase. *J. Virol.* **24:** 618–626.

———. 1977b. Identification of the herpes simplex virus DNA polymerase gene. *Nature* **269:** 621–623.

Powell, K.L., E. Littler, and D.J.M. Purifoy. 1981. Nonstructural proteins of herpes simplex virus. II. Major virus-specific DNA-binding protein. *J. Virol.* **39:** 894–902.

Purifoy, D.J.M. and K.L. Powell. 1981. Temperature-sensitive mutants in two distinct complementation groups of herpes simplex virus type 1 specify thermolabile DNA polymerase. *J. Gen. Virol.* **54:** 219–222.

Purifoy, D.J.M., R.B. Lewis, and K.L. Powell. 1977. Identification of the herpes simplex virus DNA polymerase gene. *Nature* **269:** 621–623.

Quinlan, M.P., L.B. Chen, and D.M. Knipe. 1984. The intranuclear location of a herpes simplex virus DNA-binding protein is determined by the status of viral DNA replication. *Cell* **36:** 857–868.

Rabkin, S.D. and B. Hanlon. 1990. Herpes simplex virus DNA synthesis at a preformed replication fork in vitro. *J. Virol.* **64:** 4957–4967.

Ruyechan, W.T. 1983. The major herpes simplex virus DNA-binding protein holds single-stranded DNA in an extended configuration. *J. Virol.* **46:** 661–666.

Ruyechan, W.T. and A.C. Weir. 1984. Interaction with nucleic acids and stimulation of the viral DNA polymerase by the herpes simplex virus type 1 major DNA-binding protein. *J. Virol.* **52:** 727–733.

Schaffer, P.A., M.J. Tevethia, and M. Benyesh-Melnick. 1974. Recombination between temperature-sensitive mutants of herpes simplex virus type 1. *Virology* **58:** 219–228.

Schepers, A., D. Pich, and W. Hammerschmidt. 1993a. A transcription factor with homology to the AP-1 family links RNA transcription and DNA replication in the lytic cycle of Epstein-Barr virus. *EMBO J.* **12:** 3921–3929.

Schepers, A., D. Pich, J. Mankertz, and W. Hammerschmidt. 1993b. *cis*-Acting elements in the lytic origin of DNA replication of Epstein-Barr virus. *J. Virol.* **67:** 4237–4245.

Schroder, C.H., B. Stegmann, H.F. Lauppe, and H.C. Kaerner. 1975. An unusual defective genotype derived from herpes simplex virus strain ANG. *Intervirology* **46:** 270–284.

Severini, A., A.R. Morgan, D.R. Tovell, and D.L. Tyrrell. 1994. Study of the structure of replicative intermediates of HSV-1 DNA by pulsed-field gel electrophoresis. *Virology* **200:** 428–435.

Shao, L., L.M. Rapp, and S.K. Weller. 1993. Herpes simplex virus 1 alkaline nuclease is required for efficient egress of capsids from the nucleus. *Virology* **196:** 146–162.

Sherman, G., J. Gottlieb, and M.D. Challberg. 1992. The UL8 subunit of the herpes simplex virus helicase-primase complex is required for efficient primer utilization. *J. Virol.* **66:** 4884–4892.

Skaliter, R. and I.R. Lehman. 1994. Rolling circle DNA replication in vitro by a complex of herpes simplex virus type 1-encoded enzymes. *Proc. Natl. Acad. Sci.* **91:** 10665–10669.

Smiley, J.R., M.J. Wagner, W.P. Summers, and W.C. Summers. 1980. Genetic and physical evidence for the polarity of transcription of the thymidine kinase gene of herpes

simplex virus. *Virology* **102:** 83–93.

Spaete, R.R. and N. Frenkel. 1982. The herpes simplex virus amplicon: A new eucaryotic defective-virus cloning-amplifying vector. *Cell* **30:** 295–304.

Stabell, E.C. and P.D. Olivo. 1993. A truncated herpes simplex virus origin binding protein which contains the carboxyl teminal origin binding domain binds to the origin of replication but does not alter its conformation. *Nucleic Acids Res.* **21:** 5203–5211.

Stow, N.D. 1982. Localization of an origin of DNA replication within the TRs/IRs repeated region of the herpes simplex virus type 1 genome. *EMBO J.* **1:** 863–867.

———. 1993. Sequences at the C-terminus of the herpes simplex virus type1 UL30 protein are dispensable for DNA polymerase activity but not for viral origin-dependent DNA replication. *Nucleic Acids Res.* **21:** 87–92.

Stow, N.D. and E.C. McMonagle. 1983. Characterization of the TRs/IRs origin of DNA replication of herpes simplex virus type 1. *Virology* **130:** 427–438.

Tabor, S., H.E. Huber, and C.C. Richardson. 1987. *Escherichia coli* thioredoxin confers processivity on the DNA polymerase activity of the gene 5 protein of bacteriophage T7. *J. Biol. Chem.* **262:** 16212–16223.

Tenney, D.J., W.W. Hurlburt, P. Micheletti, M. Bifano, and R.K. Hamatake. 1994. The UL8 component of the herpes simplex virus helicase-primase complex stimulates primer synthesis by a subassembly of the UL5 and UL52 components. *J. Biol. Chem.* **269:** 5030–5035.

Tenney, D.J., K. Sheaffer, W.W. Hurlburt, M. Bifano, and R. Hamatake. 1995. Sequence-dependent primer synthesis by the herpes simplex virus helicase-primase complex. *J. Biol. Chem.* **270:** 9129–9136.

Tenney, D.J., W.W. Hurlburt, M. Bifano, J.T. Stevens, P.A. Micheletti, R.K. Hamatake, and M.G. Cordingley. 1993a. Deletions of the carboxy terminus of herpes simplex virus type 1 UL42 define a conserved amino-terminal functional domain. *J. Virol.* **67:** 1959–1966.

Tenney, D.J., P.A. Micheletti, J.T. Stevens, R.K. Hamatake, J.T. Matthews, A.R. Sanchez, W.W. Hurlburt, M. Bifano, and M.G. Cordingley. 1993b. Mutations in the C-terminus of herpes simplex virus type 1 DNA polymerase can affect binding and stimulation by its accessory protein UL42 without affecting basal polymerase activity. *J. Virol.* **67:** 543–547.

Thomas, M.S., M. Gao, D.M. Knipe, and K.L. Powell. 1992. Association between the herpes simplex virus major DNA-binding protein and alkaline nuclease. *J. Virol.* **66:** 1152–1161.

Tsurumi, T. 1993. Purification and characterization of the DNA-binding activity of the Epstein-Barr virus DNA polymerase accessory protein BMRF1 gene products, as expressed in insect cells by using the baculovirus system. *J. Virol.* **67:** 1681–1687.

Tsurumi, T., T. Daikoku, and Y. Nishiyama. 1994. Further characterization of the interaction between the Epstein-Barr virus DNA polymerase catalytic subunit and its accessory subunit with regard to the 3′-to-5′ exonucleolytic activity and stability of initiation complex at primer terminus. *J. Virol.* **68:** 3354–3363.

Tsurumi, T., T. Daikoku, K. Ryutaro, and Y. Nishiyama. 1993a. Functional interaction between Epstein-Barr virus DNA polymerase catalytic subunit and its accessory subunit in vitro. *J. Virol.* **67:** 7648–7653.

Tsurumi, T., A. Kobayashi, K. Tamai, T. Daikoku, R. Kurachi, and Y. Nishiyama. 1993b. Functional expression and characterization of the Epstein-Barr virus DNA polymerase catalytic subunit. *J. Virol.* **67:** 4651–4658.

Vaughan, P.J., D.J.M. Purifoy, and K.L. Powell. 1985. DNA-binding protein associated with herpes simplex virus DNA polymerase. *J. Virol.* **53:** 501–508.

Vaughan, P.J., L.M. Banks, D.J.M. Purifoy, and K.L. Powell. 1984. Interactions between herpes simplex virus DNA-binding proteins. *J. Gen. Virol.* **65:** 2033–2041.

Vlasny, D.A. and N. Frenkel. 1982. Replication of herpes simplex virus DNA: Localization of replication recognition signals within defective virus genomes. *Proc. Natl. Acad. Sci.* **78:** 742–746.

Weber, P.C., M. Levine, and J.C. Glorioso. 1990. Recombinogenic properties of herpes simplex virus type-1 DNA sequences resident in simian virus-40 minichromosomes. *J. Virol.* **64:** 300–306.

Weber, P.C., M.D. Challberg, N.J. Nelson, M. Levine, and J.C. Glorioso. 1988. Inversion events in the HSV-1 genome are directly mediated by the viral DNA replication machinery and lack sequence specificity. *Cell* **54:** 369–381.

Weir, H.M. and N.D. Stow. 1990. Two binding sites for the herpes simplex virus type 1 UL9 protein are required for efficient activity of the oriS replication origin. *J. Gen. Virol.* **71:** 1379–1385.

Weir, H.M., J.M. Calder, and N.D. Stow. 1989. Binding of the herpes simplex virus type 1 UL9 gene product to an origin of viral DNA replication. *Nucleic Acids Res.* **17:** 1409–1425.

Weller, S.K., E.P. Carmichael, D.P. Aschman, D.J. Goldstein, and P.A. Schaffer. 1987. Genetic and phenotypic characterization of mutants in four essential genes that map to the left half of HSV-1 UL DNA. *Virology* **161:** 198–210.

Weller, S.K., A. Spadaro, J.E. Schaffer, A.W. Murray, A.M. Maxam, and P.A. Schaffer. 1985. Cloning, sequencing, and functional analysis of oriL, a herpes simplex virus type 1 origin of DNA synthesis. *Mol. Cell. Biol.* **5:** 930–942.

Werstuck, G., P. Bilan, and J.P. Capone. 1990. Enhanced infectivity of herpes simplex virus type-1 viral DNA in a cell line expressing the *trans*-inducing factor Vmw65. *J. Virol.* **64:** 984–991.

Wong, S.W. and P.A. Schaffer. 1991. Elements in the transcriptional regulatory region flanking herpes simplex virus type 1 oriS stimulate origin function. *J. Virol.* **65:** 2601–2611.

Wu, C.A., N.J. Nelson, D.J. McGeoch, and M.D. Challberg. 1988. Identification of herpes simplex virus type 1 genes required for origin-dependent DNA synthesis. *J. Virol.* **62:** 435–443.

Xu, B. and D.A. Clayton. 1995. A persistent RNA-DNA hybrid is formed during transcription at a phylogenetically conserved mitochondrial DNA sequence. *Mol. Cell. Biol.* **15:** 580–589.

Yamada, Y., N. Yamamoto, K. Maeno, and Y. Nishiyama. 1990. Role of DNA topoisomerase-I in the replication of herpes simplex virus type-2. *Arch. Virol.* **110:** 121–127.

Zhang, X., S. Efstathiou, and A. Simmons. 1994. Identification of novel herpes simplex virus replicative intermediates by field inversion gel electrophoresis: Implications for viral DNA amplification strategies. *Virology* **202:** 530–539.

Zhu, L. and S.K. Weller. 1988. UL5, a protein required for HSV DNA synthesis: Genetic analysis, overexpression in *Escherichia coli*, and generation of polyclonal antibodies. *Virology* **166:** 366–378.

———. 1992a. The UL5 gene of herpes simplex virus type 1: Isolation of a lacZ insertion mutant and association of UL5 gene product with other members of the helicase-

primase complex. *J. Virol.* **66:** 458–468.

———. 1992b. The six conserved helicase motifs of the UL5 gene product, a component of the herpes simplex virus type 1 helicase-primase, are essential for its function. *J. Virol.* **66:** 469–479.