The two helicases of herpes simplex virus type 1 (HSV-1)

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TABLE OF CONTENTS

1. ABSTRACT

Herpes simplex virus type 1 (HSV-1) encodes two helicases both of which are essential for viral DNA synthesis. UL9 binds specifically to the origins of replication and is believed to initiate DNA replication at one of three origins of replication located in the HSV-1 genome. The heterotrimeric helicase-primase complex, encoded by the UL5, UL8 and UL52 genes, is believed to unwind duplex viral DNA at replication forks and to prime lagging strand synthesis. Functional analyses of UL9 and the helicase-primase complex will be discussed with attention to the roles these proteins play during HSV-1 replication.
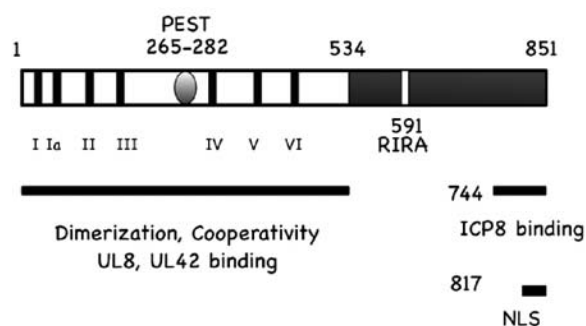
2. INTRODUCTION

Much of what we know today about helicases comes from the study of phage and viral systems primarily because these systems are amenable to genetic and biochemical analysis. The study of these viral helicases has greatly enhanced our understanding of the mechanisms of viral replication and has also provided powerful model systems for understanding the roles of helicases in cellular systems. Furthermore, several viral helicases are under consideration as potential targets for antiviral therapy (1-5). In this review, we focus on the helicases encoded by the herpesvirus family, primarily the two replicative helicases encoded by the herpes simplex virus type 1, UL5 and UL9.

3. CONSERVED MOTIFS ARE PRESENT IN ALL HELICASES

Known and putative helicases contain common conserved motifs believed to play pivotal roles in their activities (6). Based on conserved motifs, helicases have been divided into three superfamilies (SF1, SF2, and SF3), the F4 family and a fifth small family of proteins represented by the bacterial transcription termination factor rho (6, 7). All five families of helicases contain signature Walker A and B box

Figure 1. Domain structure of UL9. The UL9 gene is shown with seven black boxes depicting each of the motifs shared in SF 2 helicases. The putative PEST sequence (residues 265-282) is represented by a stippled oval. The N-terminus also contains sequences required for dimerization, cooperative binding to the origin, UL8 and UL42 binding (shown as a black box below the gene). The DNA binding C-terminal domain is depicted in black, and the residues responsible for DNA binding have been mapped between 564 and 832 residues (not shown on figure). An inactivating mutation RIRA (at residue 591) is shown below the gene. The very C-terminus contains regions involved in nuclear localization and binding to ICP8 (33, 111).

sequences, involved in NTP binding/hydrolysis (8). In this review we will focus on the helicases of Herpes Simplex Viruses, a replicative helicase (UL5) which is a member of SF1 and an origin binding protein (UL9) which is a member of SF2. SF1 and SF2 helicases are characterized by the presence of seven conserved motifs. Between these two superfamilies, motifs I and II (Walker A and Walker B, respectively) are well conserved; however, motifs Ia and III – VI exhibit very limited similarity (9). SF1 and SF2 helicases are thought to be monomeric or dimeric as opposed to the SF3, F4 and rho helicases which are generally hexameric, although some can adopt heptameric configurations (10). A large number of proteins contain the seven conserved helicase motifs characteristic of SF1 and 2; however, many of these exhibit no helicase activity whatsoever. Interestingly, a growing number of such helicase-like proteins have been shown to function in other processes such as chromatin remodeling.

Structural information is now available for several SF1 helicases including Rep from *E. coli* (11) and PcrA from *B. stearothermophilus* (12, 13) and SF2 helicases including NS3 from HCV (14, 15), UvrB from *B. caldotenax* (16) and *T. thermophilus* (17) and eIF4A from *S. cerevisiae* (18). Remarkably, considerable structural similarities between helicases from SF1 and SF2 can be observed [reviewed in (7, 19-21)]. All of these helicases contain two RecA-like domains, each composed of a beta sheet core surrounded by alpha helices. The conserved helicase motifs are positioned along a cleft that runs between the two RecA-like domains 1A and 2A, comprising an ATP binding site. These two RecA-like domains provide the motor activity. SF1 helicases encode separate domains which interrupt the 1A and 2A motor domains, designated 1B and 2B, respectively [reviewed in (7)]. These domains are primarily alpha-helical and are not

conserved. The 2B domain can be positioned in two dramatically different conformational states, open and closed: in the closed conformation, the 2B domain has rotated with respect to the other domains by approximately 120°. The 1B and 2B domains are highly variable in sequence and may play different roles in different SF1 family members: the 2B domain of PcrA is thought to bind duplex DNA (22), while in the RecB subunit of the RecBCD complex, it forms an interface with the RecC protein (23). In *E.coli* Rep, the 2B has been implicated in dimerization (11), and a recent report suggests that the 2B subdomain of Rep may also play a regulatory or autoregulatory role in Rep helicase function (24). The function of the 1B domain is not clear. The 1B and 2B domains are absent in the SF2 family helicases such as NS3 from hepatitis C and Dengue (25); instead, SF2 helicases contain an unrelated C terminal domain called domain III. In summary, although some functions have been assigned to domain 2B of SF 1 helicases, the roles of domains 1B in SF1 and domain III in SF2 helicases have not been addressed experimentally. It will be of interest to learn whether they play roles in nucleic acid binding, interaction with other proteins, or other aspects of helicase function.

HSV-1 encodes two helicases, UL9 and UL5, both of which are essential for viral DNA synthesis (reviewed in (26)). UL9 binds specifically to the origins of replication and is believed to initiate HSV DNA replication by unwinding or distorting the origin of replication. The heterotrimeric helicase-primase complex, encoded by the UL5, UL8 and UL52 genes, is believed to function as the elongation helicase at replication forks. UL9 (94-kDa) is a member of SF2 and UL5 (99kDa) is a member of the SF1 helicases. In this review we will summarize what is known about the role these proteins play in viral DNA replication and outline the important questions which still remain to be answered.

4. THE ORIGIN BINDING PROTEIN UL9

UL9 possesses the following activities: DNA-dependent nucleotide triphosphatase, 3' to 5' DNA helicase on partially single-stranded substrates, ability to form dimers in solution, and cooperative origin-specific DNA binding [reviewed in (26, 27)]. Viral mutants in the UL9 gene are defective for plaque formation and viral DNA replication suggesting that UL9 is essential for viral DNA synthesis *in vivo* (28-30). The study of temperature-sensitive (*ts*) mutants in the helicase domain of UL9 indicates that UL9 is indispensable early in HSV-1 infection but not required late in infection, once DNA synthesis has initiated (30, 31). The seven conserved helicase motifs reside in the N-terminal domain (residues 1-534) while the domain responsible for specific origin binding has been mapped to the C terminal one-third of UL9 (residues 564-832). Several other functions and motifs have been identified and are shown in Figure 1, although the functional significance of these regions is not clear.

4.1. Interactions of UL9 with other viral and cellular proteins

UL9 has been shown to interact with other viral proteins including UL29 (ICP8), the single strand binding

protein (32, 33); UL8, a member of trimeric helicase/primase complex (34); and UL42, the DNA polymerase accessory protein (35, 36). The domains responsible for some of these interactions have been identified (see legend to Figure 1). ICP8 stimulates the DNA helicase and ATPase activities of UL9 protein perhaps by increasing its processivity (32, 37, 38). ICP8 may also act as a positive regulator of UL9 by neutralizing an inhibitory region of UL9 (39). UL42 has been reported to increase the ability of UL9 to unwind short, partially duplex DNA (36) and to increase the steady-state rate of ATP hydrolysis by UL9 in the presence of fully or partially ssDNA (40). The significance of these interactions in the context of the viral DNA replication process is not known. Even more unclear is the importance of reported interactions between UL9 and cellular factors such as the 180 kDa catalytic subunit of cellular polymerase alpha primase (41), human neural F-box 42 kDa protein (NFB42 or FBX2), and cellular heat shock proteins (42-44).

4.2. UL9 is a weak helicase

Although UL9 is able to unwind DNA oligos up to 100-200 bp on its own (45, 46) and together with ICP8 can unwind up to 2 kb (32), the role of UL9 in the initiation of HSV DNA replication remains uncertain because of its inability to unwind blunt-ended linear or circular double stranded DNA containing an origin of replication, even in the presence of ICP8. Two reports suggested that oriS-containing plasmids incubated with UL9 and ICP8 may contain an unwound stem-loop structure as detected by electron microscopy (47, 48); however, unwinding of duplex DNA has not been demonstrated by other biochemical methods. Elias and colleagues have reported that upon heating and cooling oriS adopts a novel conformation referred to as oriS* resulting in the formation of a DNA hairpin with 5' and 3' tails that are partially single stranded (49-52). ATP hydrolysis is triggered by the UL9-oriS* interaction, and these authors propose that the ability of UL9 to induce this altered conformation which can be more easily unwound may be important for initiation *in vivo*. Other studies from the Lehman laboratory suggest that UL9 requires the presence of ssDNA perhaps for efficient loading and unwinding (53). It is not clear, however, how these *in vitro* studies relate to events which occur *in vivo* in infected cells, and the failure of UL9 to unwind duplex origin DNA remains a major impediment to the establishment of an *in vitro* replication system which mimics HSV DNA replication.

We have taken a genetic approach to ask whether the helicase motifs of UL9 are important biologically. Mutations in five of the seven conserved motifs inactivated the function of UL9 protein in an *in vivo* complementation assay (54), and biochemical analysis of the mutant proteins indicate that residues in the conserved helicase motifs are essential for ATPase and helicase activity but not for dimerization and DNA binding ability (55, 56). Although the genetic analyses suggest that the helicase motifs of UL9 are essential for UL9 helicase activity measured *in vitro* and are also important *in vivo* during infection, these studies do not directly answer the question of whether helicase activity itself is important *in vivo* during the initiation of viral DNA synthesis.

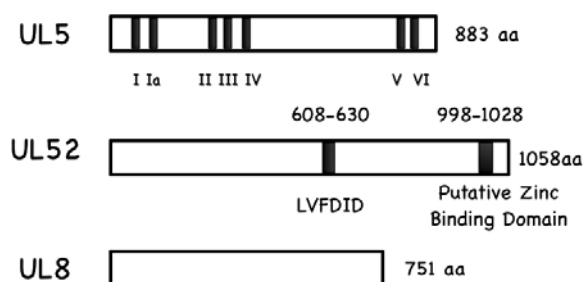### 4.3. Over expression of UL9 is inhibitory to HSV DNA replication

As mentioned above, UL9 is required during the first 6 hours of infection and is dispensable for the later stages (30, 31). In fact the over expression by transfection of either wild type UL9 protein or the C terminal origin binding domain is inhibitory to HSV-1 infection in mammalian cells (29, 57-59). Furthermore, the presence of UL9 is inhibitory to HSV DNA synthesis in insect cell lysates from cells infected with recombinant baculoviruses (60).

We and others have shown that the inhibitory effects of UL9 may be mediated by its ability to bind DNA since the inhibition could be partially relieved by an insertion of four amino acids (RIRA) in the C-terminal origin-binding domain which abrogates specific DNA binding (57-59). The deletion of the N-terminal 1-35 amino acid residues or the region between residues 292 and 404 were also able to partially relieve inhibition caused by wild type UL9 (57), suggesting that at least one other functional element in UL9 residing in the N-terminus could cause inhibition.

Various models can be considered to explain the ability of UL9 to inhibit wild type infection. Although binding at the origins is believed to be required for initiation of viral DNA synthesis, the continued presence of UL9 at one or more of the origins at later stages may prevent replication fork progression or in some other way impede the later stages of DNA replication. Although the C-terminus is believed to directly contact origin DNA, mutations in the N-terminus may exert their influence by affecting cooperative binding to the origin. This would be consistent with reports that the N-terminus is important for cooperative binding to the origin (61, 62). Alternatively, UL9 may exert its inhibitory properties through the titration of viral and cellular proteins. The N-terminus is known to contain domains required for interactions with UL8 and UL42 (34, 36, 40). The characterization of elements in the UL9 gene which affect inhibition is expected to provide insight into the role of UL9 in HSV DNA replication.

4.4. Regulation of UL9 protein levels and activity

The inhibition of infection by expression of the UL9 protein raises the possibility that the levels and/or activity of UL9 may be regulated during infection. We and others have suggested that HSV-1 replication occurs in two stages; one is UL9 dependent and the later stage is UL9 independent (26, 30, 31, 63). It is possible that UL9 activity and/or protein levels are altered during infection allowing activity during the initiation stage and inactivation and/or degradation at later stages. There is precedent in both bacteria and eukaryotic replication systems for the tight regulation of the origin binding proteins by regulation of protein levels and/or activity sometimes through post translational modification. One of the best studied systems is SV40 DNA replication in which large T antigen activity is regulated through phosphorylation (64, 65). Interestingly Isler & Schaffer reported that UL9 is phosphorylated during HSV-1 infection by either viral or virus induced cellular factors (66, 67). We note the presence of a motif in

Figure 2. The HSV-1 helicase/primase consists of three viral genes, UL5, UL8 and UL52. The UL5 gene is shown with seven black boxes depicting each of the motifs shared within SF1 members. The UL52 gene is shown with the conserved catalytic primase site and the putative Zn binding region.

the N-terminus which resembles a PEST motif (Figure 1). These motifs have been shown in other proteins to play a role in targeting proteins for ubiquitination and degradation in a phosphorylation dependent manner (68). Therefore, it will be of considerable interest to determine the role of PEST region in the regulation of UL9 protein in infection and to ask whether this region is important for the function or regulation of UL9 protein levels and activities. The suggestion that levels of UL9 are regulated is consistent with the report that UL9 can interact with NFB42, an F-box protein which is involved in polyubiquitination (43, 44). These authors suggest that since NFB42 is highly enriched in the nervous system, the interaction with UL9 may reflect a mechanism relevant to the establishment of latency. We are intrigued, however, by the possibility that the PEST region is important for regulation of UL9 levels during lytic infection.

5. THE HELICASE/PRIMASE COMPLEX UL5/8/52

The HSV-1 helicase/primase is a heterotrimeric complex composed of the products of UL5, UL8 and UL52 genes. Studies with temperature sensitive (ts) mutants (tsK13 and tsM19) and later with a null mutant (hr99) reveal that UL5 is required for DNA synthesis (69, 70). Temperature shift experiments indicate, moreover, that UL5 is required continuously for viral DNA synthesis (69, 70). UL5 was subsequently identified as part of a three-subunit complex (UL5/8/52) which possesses both helicase and primase activities (71, 72). UL5 contains seven motifs conserved in helicase superfamily 1 (Figure 2) (6, 73), thus, it is believed to be the helicase subunit of the complex. UL52 contains the conserved DXD motif involved in primase catalysis in primases (74, 75) suggesting that UL52 is the primase subunit (Figure 2). Neither UL5 nor UL52 can be expressed in an active or soluble form alone, but when they are expressed together, the subcomplex exhibits all known enzymatic activities of UL5/8/52 complex *in vitro* (76). UL8 does not show any enzymatic activities, but it stimulates the activities of the UL5/52 subcomplex (77-82). UL8 has also been shown to interact with other viral replication proteins such as the origin binding protein UL9, polymerase UL30 and single strand DNA binding protein ICP8 (34, 77-80, 83, 84), indicating a role of UL8 in protein-protein interactions at

the replication fork. In addition, UL8 has been suggested to facilitate the entry of the heterotrimer into the nucleus of infected cells (83, 84). Although it has been suggested that the three subunits of UL5/8/52 are present in the complex at a ratio of 1:1:1 (71), the stoichiometry of the active complex at a replication fork is not known.

5.1. The activities of the helicase/primase complex

The helicase/primase complex exhibits 5' to 3' DNA helicase, RNA primase and ssDNA-stimulated NTPase activities. ICP8, the HSV-1 single strand DNA binding protein has been shown to stimulate helicase activity by a mechanism that does not appear to involve increasing its processivity (79). This stimulation appears to be specific to ICP8, as it can not be achieved using the *E.coli* SSB; furthermore, the presence of UL8 is required for ICP8 to stimulate helicase activity of the UL5/52 complex (79). Since UL8 has been shown to interact with other replication proteins including ICP8, it is possible that ICP8 stimulates the helicase activity via specific protein-protein interactions through UL8.

UL5/8/52 also contains an RNA primase activity, able to carry out de novo RNA primer synthesis. The herpes primase prefers a tri-nucleotide sequence of 3'-G-pyr-pyr- 5' on the template to initiate synthesis with incorporating a purine nucleotide opposite to the pyrimidine adjacent to the G residue, while flanking sequences on the template also affects the length and the rate for primer synthesis (85, 86). Although primers of 10 to 13 nucleotides long can be synthesized, the majority of the products are only 2 or 3 nucleotides in length (85, 87). It will be of considerable interest to determine whether the herpes DNA polymerase UL30/42 can elongate from such short primers. Compared to other RNA polymerases, the HSV-1 primase has a very low fidelity with an average misincorporation rate between 1:25 and 1:60, and as high as 1:7 (87). This makes the herpes primase one of the least accurate polymerases known.

The primase may play an additional role during HSV DNA replication, that of recruitment of the DNA polymerase to the replication fork. Reminiscent of the finding that a functional primase is needed to recruit the replicative polymerase to the replication fork in *E.coli* and eukaryotic systems (88-90), two lines of evidence suggest that an active primase is required to recruit the viral DNA polymerase HSV-1 to the replication foci. First, primase defective mutants fail to carry out coupled primase-polymerase assay *in vitro* (74). Second, immunofluorescence experiments have demonstrated that primase defective mutants, but not helicase defective mutants, are unable to recruit the viral polymerase to the replication foci *in vivo* (91). It is possible that a primer is required to recruit the viral polymerase to the replication fork. Alternately, conformational changes adopted by the helicase/primase/ICP8 complex in the presence of an active primase may promote polymerase recruitment. These studies do not rule out the possibility that UL5, UL8 or the polymerase processivity factor UL42 may also play roles in polymerase recruitment. *In vitro* reconstitution assays are underway to confirm and extend these studies and to define the requirements for polymerase recruitment and initiation of viral DNA synthesis.

5.2. UL5 is the putative helicase subunit

The presence of the seven SF1 conserved motifs suggests that UL5 is likely the helicase subunit in this complex, and genetic analysis suggest that all seven motifs are essential *in vivo*. Viruses harboring mutations in the conserved amino acids in each motif of UL5 fail to complement a UL5 null mutant in a transient complementation assay (92). Wild-type and mutant versions of the UL5/52 subcomplex from insect cells infected with recombinant baculoviruses were purified and mutations in all seven of the helicase motifs exhibited severe defects in helicase activity, but wild type or higher levels of primase activity (93). Mutations in Walker A and B motifs (Motifs I and II) completely abrogated ssDNA-dependent ATPase activity (93), consistent with the notion that they are directly involved in NTPase catalysis necessary for helicase activity; however, mutations in motifs III, IV, V, and VI exhibited a moderate (3 to 6 fold) decrease in ATPase activity (93). These motifs play complex roles in the coupling of DNA-dependent ATP hydrolysis with DNA binding and unwinding of duplex DNA (94). As mentioned above, all seven conserved motifs are predicted to lie along a cleft positioned between the two recA like domains, and it is likely that they participate in communication between the ATP binding and DNA binding motifs leading to motor activity in these two subunits.

5.3. UL52 is also required for helicase activity

The UL52 subunit contains a DXD motif conserved in many primases and has been shown to be involved in primase catalysis. *In vitro* studies have shown that mutations in the DXD motif resulted in a severe defect in primase, but not helicae or ATPase activity (74, 75), indicating UL52 being the primase subunit of the helicase/primase complex. On the other hand, mounting evidence now suggests a more complex interdependence between UL5 and UL52 subunits. Sequence analyses have revealed that HSV-1 UL52 contains a putative zinc finger domain of Cys-His-Cys-Cys type at its C-terminus, which is highly conserved in many herpesvirus primases and other prokaryotic and eukaryotic primases (95, 96). Mutations of the last two conserved cysteine residues to alanine (CC34AA) resulted in viruses failing to complement the growth of a UL52 null mutant in a transient complementation assay, indicating that the UL52 zinc finger motif play an essential role for viral replication *in vivo* (97). *In vitro*, the purified subcomplex containing the wild type UL5 and UL52 harboring the CC34AA mutation exhibited defects in not only primase but also all the other known enzymatic activities of the complex (97). Cross-linking assays demonstrated that the UL5/UL52 complex containing the UL52 CC34AA mutant was unable to bind to single stranded DNA (94). These results indicate that UL52, the putative primase subunit of the helicase/primase complex, is important for more than just primase activity; rather, it appears to play a more active role in helicase and DNA binding activity than previously thought. In a study further examining the role of zinc finger motif in the helicase/primase complex, viruses containing alanine mutations from the conserved residues in the UL52 zinc finger motif showed varying abilities to complement the

growth of a UL52 null virus, ranging from totally defective to partially complementing (91). One mutant exhibited a potentiating phenotype, resulting in a higher than wild type level of complementation efficiency (Carrington-Lawrence and Weller, unpublished data). Four of the mutant complexes were purified *in vitro* from insect cells infected with recombinant baculoviruses coexpressing the wild type UL5 and UL8, as well as the mutated UL52 (98). All of the zinc finger mutants showed a severe defect in primase activity, confirming its essential role for the primase activity. Complementation defective mutants displayed greatly reduced ATPase, DNA binding and helicase activities as well. Partially complementing mutants exhibited an intermediate decrease of these activities (98). The observation that mutations in the putative zinc finger of UL52 were defective in all of the biochemical activities of UL5/8/52 confirms a complex interdependence between the UL5 and UL52 subunits. In addition, it has been shown that an intertypic HSV helicase/primase complex consisting of UL5 from HSV-2 but UL8 and UL52 from HSV-1 displayed a severe defect in primase activity and a moderate drop in helicase activity (99), again suggesting that proper interactions between the subunits of the HSV-1 helicase/primase complex is important for its full function.
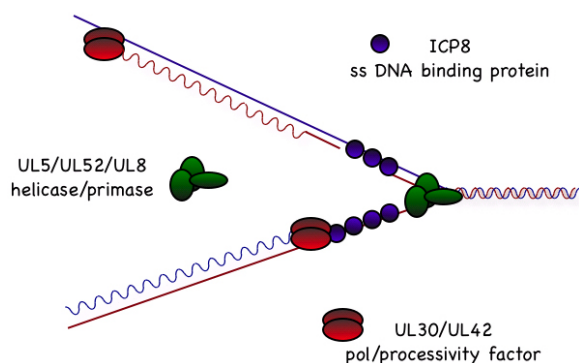
5.4. DNA binding of the UL5/8/52 complex

The initial events occurring at the origin are not well understood, but it is believed that UL9 in conjunction with ICP8 must unwind or distort the DNA to allow the helicase/primase complex to load. The mechanisms of loading is also not well understood, but it is known that the UL5/8/52 complex displays a higher affinity to forked DNA than to single stranded DNA and that it is unable to bind and unwind fully annealed double stranded linear or cruciform DNA ((97) (Chen and Weller, unpublished data). Thus, it appears that single stranded DNA is required to load the helicase/primase complex. It will be of interest to determine the minimal length of ssDNA region required for loading and to map the DNA binding site or sites on the complex. Cross-linking experiments have shown that on a forked substrate, UL5 binds at or near the junction region between the dsDNA and the ssDNA, while UL52 binds at the single stranded tail (94), consistent with their putative function in helicase and primase, respectively. Based on the observation that mutations in the UL52 zinc finger motif, but not UL5 conserved motifs resulted in DNA binding defects (93, 97, 98), it is possible that recruitment of the UL52 via its zinc finger motif to single stranded regions at the replication fork may be required to load the UL5 onto the fork junction region. Alternatively, UL5 and UL52 may share a DNA binding interface created by interactions between the two subunits. A model for how HSV replication proteins assemble at a replication fork is shown in Figure 3.

6. VIRAL HELICASES AS TARGETS FOR ANTIVIRAL CHEMOTHERAPY

HSV is a major human pathogen which can be life threatening even in immunocompetent adults. In the absence of an effective vaccine strategy against HSV and other human herpesviruses, antiviral treatments are very

Figure 3. Model for HSV DNA replication. An HSV-1 replication fork would be expected to contain the helicase-primase complex (UL5/UL52/UL8) at the fork: UL5 would be expected to unwind duplex DNA ahead of the fork and U52 would be expected to lay down RNA primers which could then be extended by the two-subunit DNA polymerase (UL30/UL42). The HSV-1 Pol would also be expected to carry out leading strand synthesis. ICP8 (UL29, SSB) would be expected to bind to ssDNA generated during HSV DNA synthesis.

important. The anti-HSV treatments currently in use are targeted to the viral polymerase, including acyclovir, penciclovir, valacyclovir and famciclovir (100). Although potent and safe, the emergence of drug resistance mutations from the polymerase inhibitors underscores the need for the development of new anti-viral strategies. Since both viral helicases are essential for viral DNA replication and infection, these proteins can be exploited as novel targets. Several strategies have been proposed, including the use of transdominant peptides which have been shown to be potent inhibitors of viral infection (101, 102). A compound, bis-peptide nucleic acid (bis-PNA), has been described which appears to inhibit the unwinding activity of UL9 (103). In addition, 1,3-phenylene derivatives have also been reported which appear to act by inhibiting the interaction of origin binding protein with the DNA (104). More progress has been made in identifying compounds which specifically inhibit the helicase-primase. Two groups of compounds, the thiazole amide derivatives like BAY57-1293 by Kleymann et al (105, 106) and the amino-thiazoly-phenyl containing drug B1LS-179-BS and its analogue (107, 108), have been reported to target the helicase/primase. Although working through different mechanisms, they are both orally active and exhibit potent antiviral effects, especially for acyclovir resistant mutants. BAY57-1293 has been reported to bind to both the UL5 and UL52 subunits of the helicase/primase, and not only inhibits both helicase and primase activity but also the subsequent viral DNA polymerase activity (106). The B1LS-179-BS inhibits the helicase, primase and DNA-dependent ATPase activities of the helicase/primase by stabilizing the interaction between the helicase/primase and the DNA template, thus preventing the linked catalytic cycles. This drug has been shown to block viral replication (107). Drug resistant mutations of these compounds have been mapped to both the UL5 and UL52 subunits (105,

106, 109), indicating that these inhibitors may bind to the helicase/primase complex at the interface shared by the two subunits. It is hoped that these promising studies will result in the development of new strategies which will be effective against drug resistant viruses.

7. SUMMARY AND FUTURE DIRECTIONS

In summary, it is clear that both UL9 and the helicase/primase complex UL5/8/52 are essential for HSV DNA replication. Furthermore, the conserved helicase motifs are essential both *in vivo* and for *in vitro* helicase activity. Many questions remain, however, about their mechanism of action at a viral replication fork. Interactions with viral and cellular proteins have been identified, however, the functional significance of most of these interactions is unclear. Although both viral helicases can bind and unwind *in vitro* substrates that contain ssDNA, neither are active on duplex DNA, raising important questions about how these helicases are loaded onto double-stranded viral DNA. It is possible that loading factors or other proteins such a chromatin remodeling agents will be necessary to facilitate the loading particularly of UL9 at an origin of replication. Identification of viral or cellular proteins which facilitate loading of UL9 onto duplex origin-containing DNA might be expected to lead to the development of an origin-dependent unwinding assay which in turn could lead to the reconstitution of origin-dependent DNA synthesis.

Our understanding of the mechanism of action of viral helicases would also be improved by structural and biophysical information. Interestingly, the herpes helicase/primase is the only known helicase in superfamily 1 that coordinates semi-discontinuous DNA replication. All other replicative helicases are hexameric or heptameric helicases which are believed to form ring structures around DNA such as DnaB or the T7 helicase/primase. As mentioned above, SF1 and SF2 helicases are in general thought to be monomeric or dimeric, and none have been shown to form rings. Whether the HSV-1 helicase/primase forms rings is a fundamental question that needs to be addressed by structural approaches. The only other SF1 or SF2 helicase known to act processively is the Rec BCD protein. The recent crystal structure of the Rec BCD complex revealed many important aspects which explain not only its processivity but also its remarkable ability to unwind both in the 3' to 5' direction and in the 5' to 3' direction as well as to act as nuclease and potent recombinase (23). Like the RecBCD helicase, the UL5/8/52 complex is a multisubunit helicase whose subunits need to be cotranslated to assemble into an enzymatically active holoenzyme. The Rec BCD crystal structure reveals extensive interaction domains between subunits which may explain the need for cotranslation to achieve proper folding. Two hybrid analysis reveals extensive interaction domains between UL5 and UL52 [(110) and (Liu and Challberg, unpublished data)]. Biophysical experiments to determine the stoichiometry of protein complexes at the origin would also be helpful. UL9 is known to form dimers in solution, but whether higher order complexes form at the origin leading to cooperative

binding is not clear. Furthermore, it will be important to determine whether the helicase/primase complex acts as a monomer of the heterotrimer or whether higher order complexes are formed at a replication fork. Mechanisms of processivity, the importance of interactions between the viral replication proteins UL9, UL5/8/52, ICP8 and the polymerase holoenzyme will also be of considerable interest and would be expected to lead to a more complete understanding of the role of these helicases in viral DNA replication. Models predicted based on structural and biophysical data can be confirmed by genetic analysis. For instance, mutations in the conserved motifs of both UL9 and UL5 have been isolated and studied, however, very little attention has been paid to other regions of these proteins, such as the 1B and 2B domains of UL5 or domain III of UL9. We anticipate that structure-function information using a combination of genetic, structural and biochemical approaches will be necessary to better understand the role of the HSV helicases in DNA replication, to elucidate their mechanism of action and to mount a more effective antiviral offensive.

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