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Specific Residues in the Connector Loop of the Human Cytomegalovirus DNA Polymerase Accessory Protein UL44 Are Crucial for Interaction with the UL54 Catalytic Subunit

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The human cytomegalovirus DNA polymerase includes an accessory protein, UL44, which has been proposed to act as a processivity factor for the catalytic subunit, UL54. How UL44 interacts with UL54 has not yet been elucidated. The crystal structure of UL44 revealed the presence of a connector loop analogous to that of the processivity subunit of herpes simplex virus DNA polymerase, UL42, which is crucial for interaction with its cognate catalytic subunit, UL30. To investigate the role of the UL44 connector loop, we replaced each of its amino acids (amino acids 129 to 140) with alanine. We then tested the effect of each substitution on the UL44-UL54 interaction by glutathione S-transferase pulldown and isothermal titration calorimetry assays, on the stimulation of UL54-mediated long-chain DNA synthesis by UL44, and on the binding of UL44 to DNA-cellulose columns. Substitutions that affected residues 133 to 136 of the connector loop measurably impaired the UL44-UL54 interaction without altering the ability of UL44 to bind DNA. One substitution, I135A, completely disrupted the binding of UL44 to UL54 and inhibited the ability of UL44 to stimulate long-chain DNA synthesis by UL54. Thus, similar to the herpes simplex virus UL30-UL42 interaction, a residue of the connector loop of the accessory subunit is crucial for UL54-UL44 interaction. However, while alteration of a polar residue of the UL42 connector loop only partially reduced binding to UL30, substitution of a hydrophobic residue of UL44 completely disrupted the UL54-UL44 interaction. This information may aid the discovery of small-molecule inhibitors of the UL44-UL54 interaction.

Replicative DNA polymerases are capable of synthesizing long stretches of DNA without dissociating from the template. The high processivity of these polymerases is dependent upon accessory proteins, called processivity factors, that bind to the catalytic subunit of the polymerase. As an example, the human cytomegalovirus (HCMV) DNA polymerase is composed of a catalytic subunit, Pol, or UL54, which possesses a basal DNA polymerase activity (4, 17), and an accessory protein, UL44 (7), which has been shown to bind double-stranded DNA, to interact specifically with UL54, and to stimulate long-chain DNA synthesis, possibly by increasing the processivity of the polymerase along the DNA template (7, 28). The details of the UL44-UL54 interaction and its role in UL44 function have not been yet completely elucidated. Recent data demonstrated that, like UL30, the catalytic subunit of herpes simplex virus type 1 (HSV-1) DNA polymerase, UL54, interacts with the cognate accessory subunit through the C-terminal region (15, 16), despite the fact that the C termini of UL54 and UL30 share almost no amino acid sequence homology. In addition, UL44 has been predicted to possess a structure with a “processivity fold” similar to that reported for the processivity subunit of HSV-1 DNA polymerase, UL42 (29). Indeed, the crystal structure of UL44 revealed an overall fold strikingly similar to that of UL42 (1), even though again the HCMV accessory protein has very little sequence homology to UL42.

The structure of UL44 includes an element analogous to the so-called connector loop, a long loop which connects the two topologically similar domains of UL42 (29) and is crucial for interaction with its cognate catalytic subunit, UL30 (2). Taken together, these observations suggested that the two subunits of HCMV DNA polymerase could interact in a way which is analogous to that of the two subunits of HSV DNA polymerase and therefore the UL44 connector loop could play a role in the UL54-UL44 interaction.

However, recent studies highlighted differences between the UL54-UL44 and UL30-UL42 interactions. A mutational analysis revealed that the UL54 residues crucial for interaction with UL44 are hydrophobic (16), whereas the UL30 residues important for UL42 binding are basic (1a, 2). Moreover, UL44 can form homodimers in both its crystal structure and in solution (1), unlike UL42, which is a monomer (8, 9, 22). Therefore, despite the remarkable structural similarities between UL44 and UL42, the UL54 binding site on UL44 might differ from the UL30 binding site on UL42.

To investigate whether the UL44 connector loop is indeed involved in the interaction with UL54, we engineered a series of substitutions in the connector loop of UL44 (amino acids 129 to 140) and tested the effect of each substitution on the physical and functional interactions between UL54 and UL44. Our findings highlight important similarities and differences between the UL54-UL44 and UL30-UL42 interactions. This might aid in the discovery of new drugs for the treatment of HCMV infection based on disruption of the UL54-UL44 interaction.
MATERIALS AND METHODS

Plasmids. The pRSET-Pol plasmid, containing the HCMV strain AD169 UL54 gene, was a gift from P. F. Ertl (GliaxSmithKline, Stevenage, United Kingdom). The pT730 plasmid, expressing HSV-1 UL30 under a T7 promoter, and the pD15-GST and pD15-UL44ΔC290 plasmids, which express glutathione S-transferase (GST) and the N-terminal 290 residues of UL44 as a GST fusion protein, respectively, have been described previously (5, 16). Plasmids pD15-UL44ΔC260, pD15-UL44ΔC315, and pD15-UL44ΔC340, which were used for the expression of GST fusion proteins with the N-terminal 260, 315, and 340 residues of UL44, respectively, were constructed in a manner analogous to that reported previously for pD15-UL44ΔC290 (16). A list of the primers used to create these constructs is available at http://coen.med.harvard.edu.

All UL44 connector loop mutants were obtained with the Quick-Change mutagenesis kit (Stratagene), amplifying the pD15-UL44ΔC290 plasmid with primer pairs containing appropriate nucleotide changes. A list of the mutagenic primers is available at http://coen.med.harvard.edu. All constructs were sequenced by the Biopolymers Laboratory in the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School to confirm the presence of the engineered mutation and the absence of undesired mutations.

Proteins and peptide. Purified baculovirus-expressed HCMV UL54, prepared as described previously (15), was generously provided by H. S. Marsden (Institute of Virology, Glasgow, United Kingdom). GST and wild-type and mutant GST-UL44ΔC290 fusion proteins were purified from *Escherichia coli* BL21 (DE3)pLysS harboring the appropriate plasmid, as described previously (16). Concentrations of all proteins were determined by amino acid analysis at the Molecular Biology Core Facility, Dana-Farber Cancer Institute.

The peptide corresponding to the last 22 residues of UL54 (here termed UL54 peptide 1, as in references 15 and 16) was synthesized by the Biopolymers Institute of Virology, Glasgow, United Kingdom). GST and wild-type and mutant GST-UL44ΔC290 fusion proteins were purified from *Escherichia coli* BL21 (DE3) pLysS harboring the appropriate plasmid, as described previously (16). Concentrations of all proteins were determined by amino acid analysis at the Molecular Biology Core Facility, Dana-Farber Cancer Institute. The peptide was then synthesized to resemble the last 22 residues of UL54 (here termed UL54 peptide 1, as in references 15 and 16) and was synthesized by the Biopolymers Laboratory in the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School. The peptide was dissolved in water, and the concentration was determined by quantitative amino acid analysis performed by the Molecular Biology Core Facility, Dana-Farber Cancer Institute.

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UL44ΔC290 bound UL54 as well as did full-length UL44, confirming that the truncated protein maintained the ability to interact with UL54. Control experiments indicated that the binding was specific, as GST did not bind UL54 and GST-UL44ΔC290 did not bind HSV-1 UL30 (Fig. 3).

We then tested the ability of UL44ΔC290 to stimulate the DNA polymerase activity of UL54 by assaying the rate of incorporation of labeled dTTP into different templates with a filter-based assay. In this assay, UL44ΔC290 stimulated the incorporation activity of UL54 with either poly(dA)-oligo(dT) (Fig. 4A) or calf thymus DNA (data not shown) as a template. Comparing different amounts of either full-length UL44 or UL44ΔC290, the stimulatory activities of the two proteins were indistinguishable. We also tested the ability of UL44ΔC290 to stimulate long-chain DNA synthesis by UL54, by measuring the incorporation of radionucleotides into an oligo(dT)/poly(dA) primer/template and analyzing the resulting products on an alkaline agarose gel (Fig. 4B). As previously observed (16), only limited synthesis of short DNA products was detectable after incubation of this template with 200 fmol of UL54 in the absence of UL44 (Fig. 4B, lanes 3 and 6), and no extension of the oligo(dT) primer was observed when only GST-UL44 or GST-UL44ΔC290 was included in the reaction (Fig. 4B, lanes 1 and 2). Stimulation of long-chain DNA synthesis was only detected when both UL54 and GST-UL44 or GST-UL44ΔC290 were included in the reaction. This stimulation was dependent on the amount of GST-UL44ΔC290 added (Fig. 4B, lanes 7 and 8), in a manner similar to that observed when equal amounts of full-length UL44 were added (lanes 4 and 5).

Taken together, these results indicate that the UL44ΔC290 truncated protein, which lacks all five C-terminal glycine strings, retains the ability to bind DNA, to interact with UL54, and to stimulate DNA synthesis by UL54. Furthermore, we observed that UL44ΔC290 exhibits less aggregation and proteolysis than the full-length protein when expressed in E. coli (data not shown).

Effects of mutations in the connector loop of UL44 on physical binding to UL54. The recent crystal structure of the UL44ΔC290 truncated protein revealed that UL44 possesses a structural element, from residues 129 to 140, analogous to the so-called connector loop of HSV-1 UL42 protein (1). This region of UL42, which stretches from residues 160 to 175, establishes specific interactions with the C terminus of UL30 (29), and substitution of a single residue in this segment, Q171, crucially affects the interaction of UL42 with UL30 (2). Therefore, the presence of a connector loop in UL44 led us to hypothesize that this structural element might play a role in the interaction with UL54.

In order to test the ability of the UL44 mutants to bind UL54, the GST pulldown assay described above was employed. We found that substitution of residue I135 with an alanine resulted in undetectable binding of UL44 to UL54 (Fig. 6). We also observed that substitutions at positions Q133, D134, and V136 of UL44 reduced binding to UL54. This was obvious for the Q133A mutant, although less obvious for the D134A and V136A mutants. However, when the ratio of bound protein to input UL44 in the two latter mutants was compared to that of wild-type protein, it was consistently lower. All of the other UL44 mutants bound UL54 in a manner similar to that of wild-type UL44.

To examine this interaction quantitatively, we used ITC, which measures heat generated or absorbed upon binding and allows one to obtain values for the stoichiometry, the dissociation constant ($K_d$), the change in enthalpy ($\Delta H$), the free energy ($\Delta G$), and the entropic term $T\Delta S$ of the interaction. We performed ITC on the interaction of wild-type and mutant GST-UL44ΔC290 with a peptide corresponding to the C-ter-
minal 22 residues of UL54, which are necessary and sufficient for interaction with UL44 (16). This peptide (termed UL54 peptide 1) has previously been shown to specifically bind to full-length GST-UL44, GST-UL44/ΔH9004/C290, and cleaved UL44/ΔH9004/C290 but not to GST alone or to a maltose-binding protein (MBP)-UL42/ΔH9004/C340 fusion which contains the N-terminal 340 residues of HSV-1 UL42 (16).

A typical titration experiment for binding of purified wild-type GST-UL44ΔC290 fusion protein to the UL54 C-terminal peptide is shown in Fig. 7. Analysis of the binding data indicated a stoichiometry of 1 molecule of peptide per molecule of GST-UL44ΔC290 fusion protein, and a \( K_d \) value of 0.7 ± 0.1 \( \mu \)M (Table 1), in agreement with the values obtained previously (16). No release of heat was detected when GST-UL44 or GST-UL44ΔC290 was titrated with a peptide corresponding to the last 10 residues of UL54 (16) (data not shown), a region previously shown not to be sufficient for binding UL44 (16). Consistent with the GST pulldown data, we observed that mutants Q133A, D134A, and V136A still bound the UL54 peptide but with affinities 4- to 10-fold lower than that of wild-type UL44 (see Fig. 7 and \( K_d \) values in Table 1). Mutant I135A, which did not detectably associate with full-length UL54 in GST pulldown assays, exhibited no measurable binding to UL54 peptide 1 (Fig. 7). As a control, we also measured the binding to UL54 peptide 1 of R137A, a UL44 mutant that does interact with full-length UL54 in GST pulldown assays. We found that this mutant possessed a \( K_d \) for the peptide (1.0 \( \mu \)M) similar to that of wild-type protein, indicating that the R137A substitution does not meaningfully affect the binding of UL44 to the C terminus of UL54.

**Mutations in the UL44 connector loop do not affect binding to DNA.** To determine whether the effects of the mutations on UL54 binding were due to global changes in protein structure, we tested the ability of UL44 mutants to bind DNA. Wild-type or mutated GST-UL44ΔC290 was expressed and labeled in vitro and subjected to DNA-cellulose chromatography as described above. As observed for wild-type UL44ΔC290, for all of the mutants, the majority of the protein was found in the

**FIG. 4. UL44ΔC290 stimulates DNA synthesis by UL54.** (A) The DNA polymerase activity of purified baculovirus-expressed UL54 alone (●) and in the presence of 200 (▲), 500 (+), or 1,000 (●) fmol of GST-UL44 (full length) or of 200 (▲), 500 (+), or 1,000 (+) fmol of GST-UL44ΔC290 was measured by incorporation of \(^{3}H\)dTTP into a poly(dA)-oligo(dT) DNA template. As a control, the activity of 1,000 fmol of GST-UL44ΔC290 alone (■) was also assayed. Samples were taken after 0, 10, 20, and 30 min of incubation at 37°C and spotted onto DE81 filters. The filters were washed, and radioactivity was counted. (B) Stimulation of UL54-mediated long-chain DNA synthesis by GST-full-length UL44 (FL) or GST-UL44ΔC290 (ΔC290) was assayed by measuring the incorporation of labeled TTP on a poly(dA)-oligo(dT) template. The reaction products were visualized by autoradiography following electrophoresis on a 4% alkaline agarose gel. Lane 1 contains 800 fmol of GST-UL44 (full-length) alone; lane 2 contains 800 fmol of GST-UL44ΔC290 alone; lanes 3 and 6 contain 200 fmol of UL54 alone; lanes 4 and 5 contain UL54 plus 400 and 800 fmol of GST-UL44 (full-length), respectively; lanes 7 and 8 contain UL54 plus 400 and 800 fmol of GST-UL44ΔC290, respectively.

**FIG. 5. UL44 mutants.** The sequence of the UL44 connector loop (amino acids 129 to 140), in single-letter code, is reported on the top. A series of substitutions were engineered in this region of UL44 as described in Materials and Methods. For each mutant, the sequence of the region containing the mutation is shown, with the mutated residue indicated by an underlined boldface letter.
fractions eluted with 500 to 1,000 mM NaCl (Fig. 8). This suggests that the mutations in the UL44 connector loop do not impair the DNA-binding activity of UL44. Moreover, the I135A mutant possessed an affinity for dsDNA similar to that of wild-type UL44C290 in both electrophoretic mobility shift assays and filter-binding assays (A. Loregian and D. M. Coen, unpublished results), indicating that this mutation does not quantitatively affect the binding of UL44 to DNA.

Since these mutants were not impaired in their DNA-binding activity, we conclude that their defect in UL54 binding is specific and not due to global misfolding of the protein.

Effect of UL44 substitutions on long-chain DNA synthesis. We next tested the ability of mutant GST-UL44C290 proteins to stimulate long-chain DNA synthesis by UL54. Of all UL44 substitutions, the I135A substitution had the greatest effect on long-chain DNA synthesis, completely abolishing the ability of UL44 to stimulate synthesis of long DNA products by UL54 (<1% of wild-type activity; Fig. 9, lanes 9 and 10).
three other mutations, Q133A, D134A, and V136A, which reduced interaction with UL54 (Fig. 6 and 7), also partially impaired long-chain DNA synthesis (Fig. 9, lanes 5 and 6, 7 and 8, and 11 and 12, respectively). When the stimulation of UL54-mediated long-chain DNA synthesis by these mutants was quantified, the Q133A, D134A, and V136A mutants were found to have about 12, 35, and 57% of wild-type UL44 activity, respectively. As a control, we assayed the ability of the R137A mutant, which interacted with UL54 in the GST pull-down and with the UL54 peptide in the ITC assays similarly to wild-type UL44, to stimulate UL54 activity. This mutant stimulated long-chain DNA synthesis as strongly as did wild-type UL44 (Fig. 9, lanes 13 and 14). Similar results were obtained by assaying the rate of incorporation of labeled dTTP into a poly(dA)-oligo(dT) template with a filter-based assay (data not shown).

The properties of selected UL44 mutants are summarized in Table 2. While the Q133A, D134A, I135A, and V136A mutants were able to bind to DNA, they were impaired for binding to UL54 and for stimulating long-chain DNA synthesis by UL54. The inhibitory effect of the Q133A, D134A, I135A, and V136A substitutions on UL44 stimulatory activity quantitatively correlates with the effect of each substitution on the binding affinity of the mutants as measured by ITC. This correlation strongly suggests the concept that the role of this segment of UL44 in stimulation of UL54 activity is via its physical interaction with UL54.

**DISCUSSION**

All known biochemical activities of UL44 as a polymerase subunit reside in the N-terminal 290 residues. The C terminus of UL44 is peculiar, as it contains several glycine-rich strings which are not present in any other human herpesvirus counterpart studied (7). A previous study suggested that this region of UL44 may be dispensable for DNA- and UL54-binding interactions.

![FIG. 8. UL44 mutants are able to bind DNA. The DNA-binding activity of UL44 mutants was tested by comparing the elution profiles of wild-type (wt) and mutant GST-UL44ΔC290 proteins and GST, as indicated to the left of the panels, from a dsDNA-cellulose column as described in the legend to Fig. 2. The concentrations of NaCl used to elute the proteins are indicated above the panels. Input, unfraccionated input protein.](http://jvi.asm.org/)
TABLE 2. Summary of the biochemical properties of selected UL44 mutants

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<td>Q133A</td>
<td>+/-</td>
<td>+/-</td>
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<td>D134A</td>
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<td>R137A</td>
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* a, wild type levels of activity; +/-, partially impaired activity; --, no detectable activity. Binding to UL54 was determined in GST pulldown experiments (Fig. 6); binding to the peptide corresponding to the C-terminal 22 residues of UL54 (UL54 peptide 1) was measured by Isothermal calorimetry (Fig. 7). DNA binding was assayed by column chromatography (Fig. 8). Stimulation of DNA synthesis by UL54 was determined both by analyzing the products on alkaline agarose gels (Fig. 9) and by filter assays (not shown).

activities and stimulation of long-chain DNA synthesis, as deletion of residues 341 to 433 or 310 to 398 of UL44 did not significantly reduce its ability to bind DNA or its capacity to stimulate UL54 (28). However, the effect of deletion of the entire C-terminal glycine-rich region of UL44 had not yet been examined. Here we show that a truncation mutant, UL44ΔC290, which lacks all five glycine-rich strings of the UL44 C terminus retains the ability to bind DNA, bind UL54, and stimulate UL54-mediated long-chain DNA synthesis in a manner similar to that of full-length UL44. This suggests that the C terminus of UL44 is not important for the role of this protein as a polymerase subunit, although it could have other, as yet unknown function(s).

Similarly, it has been shown that the C-terminal one-third of other herpesvirus processivity factors, including HSV-1 UL42, Epstein-Barr virus BMRF1, and human herpesvirus 8 PF-8, is dispensable for these proteins to bind DNA, bind their respective catalytic subunits, and stimulate long-chain DNA synthesis (3, 6, 11, 26). Consistent with these observations also is the prediction that the processivity fold, a structure shared by HSV-1 UL42 and sliding clamp processivity factors such as PCNA, is contained within the N-terminal two-thirds of these proteins (29). The recent crystal structure of UL44 has indeed shown that the N-terminal two-thirds of this protein have an overall fold strikingly similar to that of HSV-1 UL42 and of human PCNA (1).

UL54-binding site of UL44. The observation that UL44 possesses a structural element analogous to the UL42 connector loop (1) suggested that a residue(s) of this region could play a role in UL54 binding. By carrying out an extensive mutational analysis, we identified residue I135 of the UL44 connector loop as crucial for UL54 binding, as substitution of this amino acid with an alanine completely disrupted the UL54-UL44 interaction. Three other residues of the UL44 connector loop, Q133, D134, and V136, were found to participate in the interaction but with a less important role. These residues lie roughly in the middle of the connector loop, and all appear to be accessible for binding to UL54 (Fig. 10A). However, which, if any, of these residues actually interact with UL54 remains to be determined.

In contrast, although the residues of UL42 that bind to the C terminus of UL30 have been identified (29), a detailed mutational analysis to determine which of these residues are important for binding to UL30 has not yet been undertaken.

FIG. 10. Structural features of UL44. (A) The peptide backbone of UL44 is shown in grey. The connector loop of UL44 (residues 129 to 140) is highlighted in black, with the four residues identified in this study as important for binding to UL54 (Q133, D134, I135, and V136) shown as ball-and-stick models and indicated by arrows. It should be noted that the connector loop appears to be flexible, as the individual atoms display higher average temperature factors than the rest of the molecule. Thus, the side chains may adopt multiple orientations in solution, which could differ from the positions displayed in this figure. This figure was created with Molscript and Raster3D (12, 19). (B) The solvent-accessible surfaces of the face containing the connector loop of UL44 (left) and of UL42 (right) are displayed with Grasp (20). The connector loop of each protein is indicated by arrowheads. In UL42, a deep groove, which accommodates the C-terminal helix of UL30, is present between the connector loop and a projection formed by residues D63 and R64 (indicated by arrows). In contrast, the analogous region of UL44 displays a much shallower groove, as it lacks a noticeable projection below the connector loop. The corresponding position in UL44 of the UL42 projection is indicated by an arrow and an asterisk.
Initial attempts to identify regions in UL42 responsible for interaction with and stimulation of UL30 were unsuccessful (6, 21). Random peptide display studies coupled with mutational and calorimetric analyses finally identified residue Q171, which is within the UL42 connector loop, as important for binding to UL30 (2). Indeed, the Q171A substitution of UL42 drastically reduced both binding to and long-chain DNA synthesis by UL30 (2). A co-crystal structure of UL42 bound to a UL30 C-terminal peptide then showed the presence of a hydrogen-bonding network which connects Q171 to the side chain of UL30 residue R1229 (29).

Although residues of both UL44 and UL42 that are important for binding to the cognate catalytic subunit lie in the connector loop, whether the role played by these residues is similar or different in the two systems remains to be determined. Two observations hint at differences. First, weak binding of UL30 could be detected with the UL42 Q171A mutant in maltose-binding protein pulldown assays and a small release of heat was measured by ITC when a large excess of a UL30 C-terminal peptide was added to the Q171A mutant (2), whereas the I135A substitution of UL44 completely impaired binding to UL54 in GST pulldown assays and reduced the affinity for the UL54 C-terminal peptide to unquantifiable levels in ITC experiments. Second, Q171 is a polar residue, while I135 is nonpolar.

Interactions of processivity factors with cognate polymerases. The similarity in overall folding between HCMV UL44 and HSV UL42, together with the fact that residues involved in binding to the respective catalytic subunit are in the same structural element, could lead to the hypothesis that the interaction of UL44 with UL54 is analogous to that of UL42 with UL30. Moreover, the interaction of UL54 with UL44 has been mapped to the extreme C-terminus of UL54 (15), and the interaction of HSV-1 UL30 with UL42 had been mapped to the extreme C-terminus of UL30 (5, 13, 18, 24, 25). However, the molecular details of the HCMV UL54-UL44 interaction are likely to be different from those of the interaction between the HSV counterparts, as recent studies, including this report, have highlighted important differences between the two systems. In particular, the UL44-UL54 interaction is likely to be more dependent upon hydrophobic interactions, since the most important of the residues of UL44 connector loop for binding (I135) and another important residue (V136) are hydrophobic. Similarly, UL54 residues important for UL44 binding are hydrophobic (16), whereas those of UL30 important for interaction with UL42 are basic (2, 29).

Consistent with this idea are the larger $\Delta H$ values for the HCMV interaction than for the HSV-1 interaction (2, 16), which may relate to the more crucial role of hydrophobic versus hydrophilic residues in the two systems. This contrasts with the UL30-UL42 interaction, where a few specific hydrogen bonds between polar residues constitute the crucial sequence-specific determinants for binding (1a, 2).

Given the $\alpha$-helical propensity displayed by the C-terminus of UL54 (15), it is tempting to speculate that this region might adopt an $\alpha$-$\beta$-$\alpha$ structure, as seen for the analogous region of UL30 (29), and that an UL54 C-terminal $\alpha$-helix might bind in a groove of UL44, as the extreme C-terminal helix of UL30 is accommodated in a deep groove of UL42. This groove lies between the connector loop and a projection formed by residues D63 and R64 (Fig. 10B, right) (29). However, the region below the connector loop of UL44 is much flatter, as it lacks a projection analogous to that formed by D63 and R64 of UL42 (Fig. 10B) (1).

A comparison of the interaction between UL54 and UL44 with the interactions of prokaryotic and eukaryotic sliding clamps with peptides deriving from their binding partners reveals intriguing similarities. The majority of the interactions between the gp45 sliding clamp and a polymerase C-terminal peptide from bacteriophage RB69 are hydrophobic (23). Similarly, a few C-terminal residues of the cell-cycle checkpoint protein p21WAF1/CIP1 make hydrophobic interactions with human PCNA (10, 27). An alignment of the p21WAF1/CIP1 and the RB69 Pol C-terminal peptides shows that the two peptides have a very similar overall conformation, which is partially $\alpha$-helical (23). It is therefore possible that the C-terminus of UL54 folds in a manner more similar to the p21WAF1/CIP1 and RB69 Pol peptides, which bind to the same face of their partners as does the HSV UL30-derived peptide to UL42 but do not adopt an $\alpha$-$\beta$-$\alpha$ structure (10, 23). A crystal structure of UL44 bound to the C terminus of UL54 is needed to test these ideas.

The differences between HCMV and HSV DNA polymerase subunit interactions likely account for the fact that even though the residues most important for binding lie in analogous regions, i.e., the C-terminus of the catalytic subunit and the connector loop of the accessory protein, the interaction between the subunits of HSV and of HCMV DNA polymerases is specific, since noncognate partners do not bind (15).

Implications for drug discovery. New antiviral drugs are needed, especially for HCMV infections, as currently approved antivirals are frequently toxic and have pharmacological drawbacks and/or problems with viral resistance. As the interaction of UL44 with UL54 is specific and essential for long-chain DNA synthesis, it is an attractive target for antiviral compounds (14).

The demonstration that substitution of a single residue of UL44 is sufficient to completely disrupt the physical and functional interaction between UL54 and UL44 heralds the prospect that small molecules could also interfere with such interactions. Encouragement for this approach comes from the recent identification of small inhibitory molecules able to block the HSV-1 UL30-UL42 interaction in vitro as well as virus replication (21a). Thus, it is our hope that either high-throughput screening of small molecules or structure-based design will identify compounds that can specifically inhibit the UL54-UL44 interaction and HCMV replication.

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