Human cytomegalovirus UL97 is an unusual protein kinase that can phosphorylate nucleoside analogs such as ganciclovir but whose specificity for exogenous protein substrates has remained unknown. We found that purified, recombinant glutathione S-transferase-UL97 fusion protein can phosphorylate histone H2B. Phosphorylation was abrogated by substitution of glutamine for a conserved lysine in subdomain II and inhibited by a new antiviral drug, maribavir. Sequencing and mass spectrometric analyses of purified \(^{32}\)P-labeled tryptic peptides of H2B revealed that the sites of phosphorylation were, in order of extent, Ser-38, Ser-87, Ser-6, Ser-112, and Ser-124. Phosphorylation of synthetic peptides containing these sites, analyzed using a new, chimeric gel system, correlated with their phosphorylation in H2B. Phosphorylation of the Ser-38 peptide by UL97 occurred on Ser-38 and was specifically sensitive to maribavir, whereas phosphorylation of this peptide by cAMP-dependent protein kinase occurred on Ser-36. The extent of phosphorylation was greatest with peptides containing an Arg or Lys residue 5 positions downstream (P+5) from the Ser. Substitution with Ala at this position essentially eliminated activity. These results identify exogenous protein and peptide substrates of UL97, reveal an unusual dependence on the P+5 position, and may abet discovery of new inhibitors of UL97 and human cytomegalovirus replication.

Protein kinases regulate a multitude of biological processes and accordingly can act with exquisite specificity. Mechanisms for ensuring specificity include protein modules for binding specific substrates, regulation via interactions with specific ligands, phosphorylation and dephosphorylation, and sequence and structural determinants of the active site (1–3). A protein kinase with unusual substrate specificity is UL97. This enzyme, which is the product of the UL97 open reading frame of human cytomegalovirus (HCMV),\(^1\) can phosphorylate nucleoside analogues such as the antiviral drugs acyclovir and ganciclovir (4–6). UL97 is also unusual in terms of its primary sequence. It is a member of the HvUL protein kinase family, which, although sharing many motifs typical of protein kinases, is relatively divergent (7–9). Within this family, UL97 diverges even further, so that it was not immediately assumed to be a protein kinase (10). Subsequently, it was shown that UL97 can autophosphorylate on serines and threonines (11). The autophosphorylation activity has uncommon features including a rather high optimal pH (9.5), high optimal NaCl (1.5 \(\text{m}\)), and a preference for Mn\(^{2+}\) (11). However, its exogenous protein substrates and, accordingly, its specificity for such substrates have remained unknown.

UL97 is also interesting for its role in viral infection and as an antiviral drug target. Although not absolutely essential, UL97 is critical for HCMV replication in cell culture (12). There is evidence that UL97 plays roles in viral DNA synthesis, virus assembly, and egress of nucleocapsids from the nucleus (13, 14).\(^2\) A homologous kinase from another herpesvirus, herpes simplex virus, is important, at least in certain cells, for viral gene expression (15). UL97 is a target for antiviral drugs, both via its ability to activate nucleoside analogs such as ganciclovir (4–6) and as a target for inhibition. Recently, a new antiviral drug, maribavir, has been shown to be a potent and selective inhibitor of HCMV replication in cell culture (13) and in human subjects.\(^3\) Maribavir is a specific inhibitor of UL97 protein kinase activity (this report),\(^4\) and indeed its anti-HCMV activity is due to its effects on UL97\(^2,3\). Thus, UL97 is a target for both established and promising new antiviral compounds.

To investigate the specificity of UL97 for exogenous protein and peptide substrates, we first identified an exogenous protein substrate for this enzyme, calf thymus histone H2B, and then mapped the sites of phosphorylation to specific serine residues. Peptides containing these sites were synthesized and, using a new, chimeric electrophoresis technique, were assayed as substrates for UL97. One of these peptides was phosphorylated extensively by both UL97 and cyclic AMP-dependent protein kinase (PKA) but with utilization of different serines and with susceptibility to different inhibitors. Remarkably, the extent of...
phosphorylation of peptides depended critically on the residue lying 5 positions downstream of the serine. Our results identify protein and peptide substrates of UL97 that could abet assays for novel inhibitors and reveal new information regarding its unusual substrate specificity, both of which could aid in the discovery of new anti-HCMV drugs.

EXPERIMENTAL PROCEDURES

Cell and Viruses—Spodoptera frugiperda 9 cells (S9), obtained from the American Type Culture Collection, were maintained in Grace’s insect medium (Invitrogen) supplemented with 10% fetal bovine serum (Atlanta Biologicals) and 100 units/ml penicillin and 100 μg/ml streptomycin. Recombinant baculoviruses expressing wild type or mutant K355Q–HCMV UL97K355Q—All column materials were purchased from Amersham Biosciences. For initial studies, baculovirus-expressed GST-UL97 and GST-UL97K355Q were purified as described previously (11). Subsequently, to eliminate a contaminating protein kinase activity that was active on certain substrates, the previous protocol was modified as follows. The protein that eluted from a glutathione-Sepharose column was bound to a Q-Sepharose column, previously equilibrated with elution buffer (EB; 50 mM Tris, pH 8.0, 2 mM dithiothreitol, 2 mM EDTA, 10% glycerol) with 50 mM NaCl (EB-50). The Q-Sepharose was washed and maintained using standard methods (16).

Compounds—Maribavir (5,6-dichloro-2-(isopropylamino)-1-β-D-ribofuranosyl-1H-benzimidazole, also known as 1283W94) was synthesized and kindly provided by GlaxoSmithKline. Unless otherwise noted, all other chemicals were purchased from Sigma.

Purification of Baculovirus-expressed GST-UL97 and GST-UL97K355Q—All column materials were purchased from Amersham Biosciences. For initial studies, baculovirus-expressed GST-UL97 and GST-UL97K355Q were purified as described previously (11). Subsequently, to eliminate a contaminating protein kinase activity that was active on certain substrates, the previous protocol was modified as follows. The protein that eluted from a glutathione-Sepharose column was bound to a Q-Sepharose column, previously equilibrated with elution buffer (EB; 50 mM Tris, pH 8.0, 2 mM dithiothreitol, 2 mM EDTA, 10% glycerol) with 50 mM NaCl (EB-50). The Q-Sepharose was washed with 30 column volumes of EB-50 and 30 column volumes of EB-150 (EB with 150 mM NaCl), and protein was eluted with EB-50. This material was loaded onto a phenyl-Sepharose column (equilibrated with EB-1000), and the column was washed with 30 column volumes of EB-50 and 10 volumes of EB-300 with 0.1% Triton X-100. GST-UL97 was eluted with EB-0 with 0.1% Triton X-100, concentrated with a Centricon-30 concentrator, and dialyzed for 2 h against EB-50. The resulting protein was apparently homogeneous based on SDS-PAGE. Protein concentration was determined by amino acid analysis performed by Angelo Dickerson at the Molecular Biology Core Facility of the Dana-Farber Cancer Institute, and the protein was stored in aliquots at −80 °C.

Protein Kinase Assays—For phosphorylation of calf thymus histone H2B by UL97, assays were performed in 50 mM Tris-HCl (pH 9.0), 300 mM NaCl, 10 mM MgCl₂, 2 mM dithiothreitol, and 100 μM ATP, with 5 μg of a calf thymus histone protein kinase. Of these, the more faintly labeled species between lane 1 (C) for 1 h, unless otherwise specified, and terminated

RESULTS

Phosphorylation of Histone H2B by UL97 Protein Kinase—HCMV UL97 is an unusual protein kinase that autophosphorylates on serine and threonine (11). To find exogenous substrates of UL97 and study its substrate specificity, we tested a number of common substrates of protein kinases, including these histones H1, H2B, and H3 and histone mixtures were most robustly phosphorylated by purified GST-UL97. To investigate the specificity of this phosphorylation further, we initially tested the phosphorylation of calf thymus histone H2B in low salt (<5 mM NaCl). Under these conditions (Fig. 1, lane 1), H2B was efficiently radiolabeled in the presence of [γ-32P]ATP and GST-UL97 (which also became radiolabeled due to auto-phosphorylation; the more faintly labeled species between GST-UL97 and H2B co-migrates with histone H1 and is prob.

4 M. C. Baek, P. M. Kroacky, Z. W. He, and D. M. Coen, data not shown.

Identifying the Specificity of Phosphorylation of H2B by UL97 Protein Kinase—Histone H2B was efficiently radiolabeled in the presence of [γ-32P]ATP and GST-UL97 (which also became radiolabeled due to auto-phosphorylation; the more faintly labeled species between GST-UL97 and H2B co-migrates with histone H1 and is prob.
ably a contaminant of the commercial H2B preparation. Much less phosphorylation of either H2B or GST-UL97 was observed in the presence of maribavir (lane 2), a selective inhibitor of UL97 kinase activity* (see below). Similarly, much less radiolabeling was observed with a mutant form of GST-UL97 (K355Q) that contains glutamine instead of lysine at residue 355 (lane 3). Lys-355 corresponds to the invariant lysine in subdomain II of protein kinases that is crucial for enzyme activity (22), and this substitution eliminates autophosphorylation activity (11) (Fig. 1A, lane 3). These results indicated that most of the phosphorylation of H2B in Fig. 1A was due to GST-UL97 and not a contaminant in the enzyme preparation. However, neither maribavir, the K355Q mutation, nor both (lane 4) completely eliminated radiolabeling of H2B. This residual phosphorylation was also observed when H2B was incubated in kinase buffer in the absence of GST-UL97 (Fig. 1B, lanes 1 and 2), indicating that the commercial preparation of histone H2B used in these experiments contains small amounts of a contaminating kinase. When the NaCl concentration was increased to 300 mM, the residual radiolabeling in the absence of GST-UL97 was eliminated (Fig. 1B, lane 3, and Fig. 1C, lane 2), and phosphorylation by GST-UL97 was eliminated by the K355Q substitution and by maribavir (Fig. 1C, lanes 3 and 4). Under these conditions, H2B phosphorylation by GST-UL97 remained strong (Fig. 1C, lane 1), being reduced only about 20% (Fig. 1D). Higher concentrations of NaCl, up to 1 M, reduced but did not eliminate H2B phosphorylation by GST-UL97 (Fig. 1D) and stimulated autophosphorylation (11).4 Subsequent experiments with H2B were performed at 300 mM NaCl.

**Stoichiometry of H2B Phosphorylation**—To determine the stoichiometry of H2B phosphorylation, a time course study was performed (Fig. 2). After incubation at 37 °C for 1 h, the incorporation of phosphate reached a maximum of nearly 2 mol of phosphate/mol of H2B. The addition of more ATP or more enzyme after the reaction had reached this value did not increase the incorporation of phosphate,4 indicating that the phosphorylation of H2B by UL97 was complete.

**Analysis of Phosphorylated Sites in H2B**—UL97 is known to autophosphorylate on serines and threonines (11). To identify the amino acid(s) of H2B phosphorylated by GST-UL97,32P-labeled H2B was hydrolyzed, and the resulting amino acid mixture was separated by thin layer electrophoresis. Essentially all the radioactive amino acids co-migrated with phosphoserine (P-Ser), phosphothreonine (P-Thr), and phosphotyrosine (P-Tyr) standards. The positions of the phosphoamino acid standards were visualized with ninhydrin staining (as indicated to the right), and the position of the radiolabeled peptide was visualized by autoradiography. B, tryptic phosphopeptides. H2B was phosphorylated by GST-UL97, gel-purified, and digested with TPCK-trypsin. The resulting phosphopeptides were resolved on a 40% alkaline acrylamide gel and visualized by autoradiography. The peptides were designated TP1–TP5 as indicated to the right.

**Phosphoamino acid and phosphopeptide analysis.** A, phosphoamino acid analysis. H2B was phosphorylated by GST-UL97, gel-purified, hydrolyzed, and subjected to thin layer electrophoresis with phosphoserine (P-Ser), phosphothreonine (P-Thr), and phosphotyrosine (P-Tyr) standards. The positions of the phosphoamino acid standards were visualized with ninhydrin staining (as indicated to the right), and the position of the radiolabeled peptide was visualized by autoradiography. B, tryptic phosphopeptides. H2B was phosphorylated by GST-UL97, gel-purified, and digested with TPCK-trypsin. The resulting phosphopeptides were resolved on a 40% alkaline acrylamide gel and visualized by autoradiography. The peptides were designated TP1–TP5 as indicated to the right.

**Fig. 3. Phosphoamino acid and phosphopeptide analysis.** A, phosphoamino acid analysis. H2B was phosphorylated by GST-UL97, gel-purified, hydrolyzed, and subjected to thin layer electrophoresis with phosphoserine (P-Ser), phosphothreonine (P-Thr), and phosphotyrosine (P-Tyr) standards. The positions of the phosphoamino acid standards were visualized with ninhydrin staining (as indicated to the right), and the position of the radiolabeled peptide was visualized by autoradiography. B, trytic phosphopeptides. H2B was phosphorylated by GST-UL97, gel-purified, and digested with TPCK-trypsin. The resulting phosphopeptides were resolved on a 40% alkaline acrylamide gel and visualized by autoradiography. The peptides were designated TP1–TP5 as indicated to the right.

**Fig. 1. Phosphorylation of histone H2B by UL97.** A, phosphorylation at low salt. Phosphorylation of 600 ng of H2B by UL97 was performed for 30 min using 50 ng of wild type GST-UL97 (lanes 1 and 2) or mutant K355Q GST-UL97 (lanes 3 and 4) with no NaCl in the assay buffer and either in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 3 μM maribavir. The positions of GST-UL97 and H2B are indicated. B, suppression of contaminating kinase activity by high salt. The amounts of H2B indicated at the top of B were incubated in the absence of UL97 under kinase assay conditions either in the absence of NaCl (lanes 1 and 2) or in 300 mM NaCl. C, phosphorylation in 300 mM NaCl. Phosphorylation of 600 ng of H2B was performed in the presence of 40 ng of wild type GST-UL97 (lanes 1 and 3) or mutant K355Q GST-UL97 (lane 4) or in the absence of added UL97 (lane 2). D, effect of NaCl on H2B phosphorylation. 600 ng of H2B was phosphorylated by 50 ng of GST-UL97 at various concentrations of NaCl, and the extent of phosphorylation of H2B after 30 min was quantified.

**Fig. 2. Time course and stoichiometry of H2B phosphorylation.** H2B (1 μg) was phosphorylated by GST-UL97 (100 ng) in the presence of 1 mM ATP, with aliquots assayed for H2B phosphorylation at the indicated times, and the moles of radiolabel incorporated per mole of H2B incorporated were determined.
these five species was excised, eluted, and purified by RP-HPLC. Single peaks of radioactivity were detected for TP1–TP4, whereas three peaks of radioactivity were found for TP5 (TP5-A, TP5-B, and TP5-C). Each radioactive HPLC fraction was sequenced by Edman degradation, and a portion of the eluant from each cycle was collected and monitored for radioactivity to identify which residue was phosphorylated (Fig. 4).

Sequence data consistent with a single phosphorylated species were obtained for all fractions except TP2, which yielded data consistent with a major species and minor species, both of which contained a serine residue at position 6. To determine which of these two species was phosphorylated or whether both were, the TP2 fraction was analyzed by MALDI-TOF. The spectrum contained a species with a mass corresponding to a

Table I
Summary of HPLC from each peptide band and mass spectrometric analysis of radioactive HPLC fractions

| Band | HPLC fraction | Radioactivity | Mass | Predicted peptide from H2B sequence |
|------|---------------|---------------|------|-----------------------------------|
| TP1  | 37 cpmin      | 12,000        | 1345.7 | 34KESYSVYVYK34+phosphate          |
| TP2  | 23            | 4,000         | 703   | 73AGEASR73                        |
| TP3  | 38            | 12,500        | 1173  | 1PEPAKSAAPPK11+phosphate          |
| TP4  | 15            | 14,000        | 744.7 | 35ESYSVYVYK35+phosphate           |
| TP5-A| 13            | 6,000         | 908.5 | 87STITSSR87+phosphate             |
| TP5-B| 11            | 5,500         | 650.7 | 109HAVSEGTK109+phosphate          |
| TP5-C| 9             | 4,000         | 666.2 | 121YTSSK121+phosphate             |

*Cerenkov counting from the each radioactive HPLC fraction.

*Masses for peaks determined by (MALDI-TOF).

*The tryptic fragments of H2B derived from peptide masses using program MS-Digest (ProteinProspector Version 3.2.1) available on the World Wide Web at http://prospector.ucsf.edu.
monophosphorylated form of the major species predicted from the H2B sequence (accession number 70719), but not the minor species, and one corresponding to a nonphosphorylated form of the minor species but not the major species (Table 1). The MALDI-TOF spectrum of each of the other HPLC fractions contained one species with a mass corresponding to a monophosphorylated form of the sequenced peptide (Table 1).

Taken together, these results identify five different serine residues as sites of phosphate incorporation: Ser-6 (TP2 and TP4, the latter being the complete digestion product), Ser-38 (TP1 and TP3, the latter being the complete digestion product), Ser-87 (TP4), Ser-112 (TP5-A), and Ser-124 (TP5-C). These results are consistent with the results of phosphoamino acid analysis (Fig. 3A) that showed only phosphoserine. The five sites were utilized to differing extents. Ser-38 (42% of the radioactivity), Ser-87 (24%), and Ser-6 (16%) were the three most utilized by UL97, whereas Ser-112 and Ser-124 contained only 10% and 7% of the radioactivity, respectively (Table 1). The relatively weak phosphorylation at certain sites accounts for the discrepancy between the 2 mol of phosphate incorporated/mol of H2B and the five phosphorylation sites detected. Interestingly, the Ser-38, Ser-87, and Ser-6 sites contain diverse residues at all positions around the phosphorylated serine except at the position 5 residues downstream (P+5), where all three peptides contain a basic residue (Lys or Arg).

**Phosphorylation of Synthetic Peptides**—Peptides corresponding to portions of H2B containing each of the five phosphorylated serines (SP-6, SP-38, SP-87, SP-112, and SP-124) were synthesized, phosphorylated by UL97, and resolved on chimeric polyacrylamide gels, and phosphorylated species were visualized by autoradiography. The positions of labeled kinases and peptide are indicated to the left. B, sequence analysis. SP-38 phosphorylated by PKA (left panel) or GST-UL97 (right panel) was subjected to automated sequencing as in Fig. 4. The phosphorylated amino acid is indicated in boldface type. (SP-124 lacks residues beyond 1 position downstream of the phosphorylation site, because it corresponds to the C terminus of H2B.)

**Fig. 5.** Phosphorylation of H2B-derived peptides. A, peptides (SP-6, -38, -87, -112, and -124 as indicated at the top) containing one species with a mass corresponding to a monophosphorylated form of the sequenced peptide, and one corresponding to a nonphosphorylated form of the minor species but not the major species (Table 1). The MALDI-TOF spectrum of each of the other HPLC fractions contained one species with a mass corresponding to a monophosphorylated form of the sequenced peptide (Table 1).

**Fig. 6.** Specificity of phosphorylation of SP-38. A, either no peptide (lanes 1 and 5) or SP-38 (lanes 2–4 and 6–9) was incubated in the appropriate kinase buffer with either PKA (lanes 1–4), GST-UL97 (lanes 5–8), or GST-UL97K355Q (lane 9), either in the absence of inhibitors (lanes 1, 4, 5, 8, and 9) or in the presence of 5 μM PKI (lanes 2 and 6) or 3 μM maribavir (lanes 3 and 7). The phosphorylation reactions were analyzed on chimeric polyacrylamide gels, and phosphorylated species were visualized by autoradiography. The positions of labeled kinases and peptide are indicated to the left. B, sequence analysis. SP-38 phosphorylated by PKA (left panel) or GST-UL97 (right panel) was subjected to automated sequencing as in Fig. 4. The phosphorylated amino acid is indicated in boldface type.
not UL97, was sensitive to PKA, a specific pseudosubstrate inhibitor of PKA (27, 28). Moreover, phosphorylation of SP-38 by UL97 was sensitive to the K355Q substitution. When the phosphorylated peptides were analyzed by MALDI-TOF, only monophosphorylated forms were observed.\(^4\) Sequence analysis revealed that PKA phosphorylated the peptide specifically on Ser-36 and UL97 phosphorylated the peptide specifically on Ser-38, as predicted (Fig. 6B). Thus, presented with the same peptide, UL97 and PKA each have strong specificity for a particular serine as well as distinct inhibitor specificities.

**Importance of the P+5 Position**—As noted above, the three sites in H2B and the two H2B-derived peptides most extensively phosphorylated by UL97 contain a Lys or Arg in the P+5 position. Interestingly, the site on UL97 that is most extensively autophosphorylated by UL97\(^5\) also contains a basic residue at P+5 (Fig. 5B). The results raised the possibility that the rather distal P+5 position might be important for phosphorylation by UL97. To test this hypothesis, synthetic peptides derived from the two most extensively phosphorylated sites in H2B and the most extensively autophosphorylated site in UL97,\(^6\) but containing an Ala in place of Arg or Lys at the P+5 position, were synthesized. Phosphorylation of these mutant peptides was essentially eliminated, compared with their wild type counterparts (Fig. 7). Thus, phosphorylation of three different peptides by UL97 critically depends on the identity of the residue at the P+5 position.

**DISCUSSION**

The work described here documents the phosphorylation by purified HCMV UL97 of an exogenous substrate, histone H2B, and of H2B-derived peptides on specific Ser residues. To show conclusively that phosphorylation was performed by UL97 rather than by a contaminant in the enzyme or H2B preparation, we not only purified the enzyme extensively but also used both a catalytically inactive, mutant form of UL97 (K355Q) and a specific UL97 inhibitor, maribavir. HCMV UL97 is a member of the HvU\(_1\) protein kinase family (7, 10). It has been reported that certain members of this family are capable of phosphorylating various exogenous substrates in vitro, including certain preparations of histones (8, 29–33). However, in these reports, the enzymes were not purified extensively and/or it was not shown that an inactivating point mutation or a specific inhibitor eliminated phosphorylation of the exogenous substrates. Indeed, in two cases (32, 33), mutant forms akin to UL97 K355Q retained substantial kinase activity, suggesting that the enzyme preparations were contaminated with other kinases. Regardless, to our knowledge, the work presented here is the first to demonstrate phosphorylation of an exogenous substrate by UL97, an especially divergent member of this already divergent family of protein kinases, and the first to describe sites of phosphorylation by any member of this family.

Aside from H2B, UL97 can phosphorylate histones H3 and H1 in vitro.\(^4\) It is not inconceivable that histones could be physiologically relevant substrates of UL97. UL97 is localized to the nucleus in HCMV-infected cells (34, 35). HCMV infection is known to affect host gene expression (reviewed in Ref. 36), and other herpesviruses are known to affect chromatin condensation (reviewed in Ref. 37), both processes that can be regulated by histone phosphorylation (38). These possibilities can be tested with the aid of maribavir and UL97 deletion mutants (12).

A goal of this study was to find clues to the specificity of phosphorylation by UL97, both to understand the mechanism of specificity and to help predict intracellular substrates of this enzyme. Sites of phosphorylation of HvU\(_1\) protein kinases have not previously been identified. We anticipated that peptide and protein substrates of UL97 might have unusual characteristics based on its ability to phosphorylate nucleoside analogues, its sequence divergence from protein kinases, and its high pH and NaCl optima. That the specificity of UL97 differs from that of a prototypic protein kinase, PKA, is clearly illustrated by the phosphorylation of different Ser residues when both kinases are presented with the same peptide (SP-38). The phosphorylation of the Ser corresponding to Ser-36 by PKA is consistent with the established consensus substrate for PKA, (R/K)XXS (39–41).

More generally, for diverse protein kinases, specific residues from P-3 through P+3 are most important for substrate specificity, with the P-3 (e.g. PKA) and P+1 positions usually being the most crucial; it is highly unusual for the P+5 position to be important (42). Our results reveal, however, that the sites that are most extensively phosphorylated by UL97 contain an Arg or Lys at the P+5 position. Indeed, substitution of a P+5 Arg or Lys with Ala essentially eliminates phosphorylation of synthetic peptides. Thus, this rather distal residue is very important for UL97. In contrast, there were no obvious similarities in other positions of extensively phosphorylated peptides (Fig. 5B). The unusual importance of the P+5 position is consistent with the lack of conservation of UL97 with other protein kinases in subdomains typically involved in substrate recognition. In particular, UL97 lacks acidic residues in subdomains V and VI that are thought to stabilize binding of basic P-3 residues, and it does not have a readily recognizable subdomain VIII, which ordinarily makes up much of the P+1 binding site of other kinases (10, 22, 42). At present, little is known about residues in any protein kinase that interact with the P+5 position. Further studies of the substrate specificity of UL97 should advance our understanding of this unusual kinase-substrate interaction.

Our results may aid the discovery of new antiviral drugs that target UL97. The identification of exogenous substrates for UL97 permits development of assays for new inhibitors of UL97. It is especially advantageous to have identified a peptide substrate of UL97, since this can facilitate the development of high throughput assays. Moreover, peptide substrates can be the starting points for the design of peptidomimetic inhibitors of protein kinases. Such substrates can be abetted by combinatorial approaches to optimize affinity and conversion of serines to nonphosphorylatable moieties (43), followed by conversion of peptides to peptidomimetics, as has occurred in the development of protease inhibitor drugs (44). The peptide substrate, SP-38, may serve as a starting point for a novel UL97 inhibitor.

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\(^5\) M. C. Baek, P. M. Krosky, and D. M. Coen, manuscript in preparation.
