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Function of human cytomegalovirus UL97 kinase in viral infection and its inhibition by maribavir

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Summary

The serine/threonine kinase expressed by human cytomegalovirus from gene UL97 phosphorylates the antiviral drug ganciclovir, but its biological function is the phosphorylation of its natural viral and cellular protein substrates which affect viral replication at many levels. The UL97 kinase null phenotype is therefore complex, as is the mechanism of action of maribavir, a highly specific inhibitor of its enzymatic activity. Studies that utilise the drug corroborate results from genetic approaches and together have elucidated many functions of the UL97 kinase that are critical for viral replication. The kinase phosphorylates eukaryotic elongation factor 1delta, the carboxyl terminal domain of the large subunit of RNA polymerase II, the retinoblastoma tumour suppressor and lamins A and C. Each of these is also phosphorylated and regulated by cdc2/cyclin-dependent kinase 1, suggesting that the viral kinase may perform a similar function. These and other activities of the UL97 kinase appear to stimulate the cell cycle to support viral DNA synthesis, enhance the expression of viral genes, promote virion morphogenesis and facilitate the egress of mature capsids from the nucleus. In the absence of UL97 kinase activity, viral DNA synthesis is inefficient and structural proteins are sequestered in nuclear aggresomes, reducing the efficiency of virion morphogenesis. Mature capsids that do form fail to egress the nucleus as the nuclear lamina are not dispersed by the kinase. The critical functions performed by the UL97 kinase illustrate its importance in viral replication and confirm that the kinase is a target for the development of antiviral therapies.

Introduction

Human cytomegalovirus (HCMV) encodes a serine/threonine (ser/thr) protein kinase that shares common features with homologs in other herpesviruses, yet has a number of distinctive characteristics. Studies have focused on the UL97 kinase because its activity can be exploited by antiviral drugs for the treatment of HCMV infections. It was identified initially as the enzyme that activates ganciclovir (GCV) in HCMV infected cells and this drug remains the therapy of choice for these infections. But, equally important, is the development of maribavir (MBV), a potent inhibitor of HCMV replication that inhibits the enzymatic activity of the UL97 kinase and has established a successful new strategy for the therapy of viral infections. Results from pharmacologic studies with this highly specific inhibitor support those obtained from genetic approaches and together provide both a clear and comprehensive view of the myriad functions of the kinase in viral infection. Recent reports have revealed unexpected aspects of UL97 kinase function and have elucidated how this enzyme performs functions similar to cdc2/cyclin-dependent kinase 1 (cdk1) and regulates critical events in viral replication including virion morphogenesis, degradation of

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nuclear lamina and modification of the cell cycle. This review summarises common activities shared among homologs in other herpesviruses and focuses on the enzymatic and biological properties unique to the HCMV UL97 kinase. Implications of the available data are discussed and a summary of its role in viral replication is described to the extent that it is currently understood. The recognition of critical functions performed by this enzyme is important because it impacts the development of antiviral therapies for the treatment of HCMV infections and can be used to guide future strategies in many other viruses.

Common Functions of Herpesvirus Protein Kinases

While the UL97 kinase exhibits many distinct activities and structural features, it also appears to share some common characteristicts with homologs in other herpesviruses including their significant presence as virion tegument proteins [1–4]. Each of the human herpesviruses encodes at least one well-conserved ser/thr protein kinase that is homologous to pUL97 and has been reviewed previously [5–7]. These enzymes were identified initially by conserved aa motifs found in the catalytic domains of many protein kinases [8–13], and two classes of protein kinases with distinct activities are encoded by the human herpesvirus. Members of HvU_S class are encoded by the alphaherpesviruses and include the HSV US3 kinase and the VZV ORF66 kinase; neither appears to impact viral replication to a significant extent [14,15]. In contrast, all the human herpesviruses encode members of the HvU_L protein kinase family [16], which includes HSV UL13 [17], VZV ORF47 [18], EBV BGLF4 [10], HCMV UL97 [19,20], HHV-6 U69 [21], HHV-7 U69 [12] and HHV-8 ORF36 [22]. All the herpesvirus kinases share significant aa similarity [6,8,23,24], but the limited identity of the aa sequences suggests that their functions diverged early in the evolution of these viruses [6]. Although none of the enzymes is essential for replication, recombinant viruses lacking HSV UL13 and UL97 replicate poorly in some cell lines [17,25], and those lacking VZV ORF 47 and HSV UL13 replicate poorly *in vivo* [26,27], indicating that the HvU_L homologs perform important functions in the life cycle of these viruses.

A few common features of the HvU_L protein kinases are beginning to emerge including autophosphorylation, which occurs with all of these kinases and is presumed to be involved in the regulation of their activity [1,10,21,28,29]. Viral proteins are also common targets and many have been described for HSV [30–32], VZV [33,34], EBV [10,35], HCMV [36–38] and HHV-8 [39]. The DNA polymerase processivity factors encoded by each of the herpesviruses appear to be a conserved target. In cells infected with HCMV or EBV, these proteins appear to be phosphorylated directly by UL97 and BGLF4, respectively [35,37,38]. The UL13 kinase in HSV does this indirectly by activating cdk1, which in turn phosphorylates UL42 [40], in a process that also appears to involve cdc25 [41]. It is unclear if this phosphorylation impacts DNA replication, but it is reminiscent of the mechanism used to regulate proliferating cell nuclear antigen binding factors by cdk1 and cdk2 [42,43].

Cellular proteins phosphorylated by cdk1 also appear to be common targets of these kinases. HSV UL13, EBV BGLF4 and HCMV UL97 all phosphorylate elongation factor 1delta (EF-1) that is also a target of cdk1 [23,44,45]. In addition, both HSV UL13 and HCMV pUL97 phosphorylate the carboxyl-terminal domain of RNA polymerase II [46–48], which is also regulated by phosphorylation by cdk1 [49,50]. HSV UL13 is also involved in the activation of cdk1 and promotes events leading to the expression of late viral genes [51,52]. So, in addition to targeting similar substrates, UL13 also appears to redirect the activity of cdk1 and reinforces the significance of the relationship between these two kinases. The histone protein H2B can also be phosphorylated by many of these viral kinases, but its biological significance is unclear [10,21,53,54].

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Despite the biological activities common to many of these kinases, each has evolved unique functions and there appears to be limited functional complementation among them. Insertion of *UL97* in the HSV genome could partially complement the phenotype of a UL13 null mutant [55]; however, UL13 expressed from an adenovirus vector was unable to complement the replication of a UL97 deletion mutant RCΔ97 [23]. Similar studies with the adenovirus vectors showed that the rat cytomegalovirus homolog, R97, could complement plaque formation of RCΔ97, as could EBV BGLF4, albeit at a reduced efficiency [23]. Deletion of the M97 kinase gene from the murine cytomegalovirus (MCMV) genome severely impacts viral replication, yet its replacement with the *UL97* gene only partially restored its replication in the lungs and salivary glands of the mouse and did not restore the growth defects in cell culture [56]. Guinea pig cytomegalovirus also encodes a UL97 homolog [57]. Deletion of the gene encoding this kinase results in significant replication deficits in cell culture and its replacement with the *UL*97 gene appeared to restore its replication to levels that approached that of the wt virus [58]. Additional studies will help define functional complementation among UL97 homologs, but most of the available data suggest that it will be limited and confirms that their functions have diverged during evolution.

Expression and Physical Characteristics of UL97 Kinase

The *UL*97 gene is encoded as part of a large transcriptional unit that is well conserved in all of the herpesviruses [59,60]. It is transcribed early in infection from a promoter with E2F and ATF/CRE elements as a 4.7 kb transcript within a set of nested 3′ co-terminal transcripts that express each of the genes from *UL92* through *UL99* [61]. Only the first ATG is used in translation and yields a 707 aa protein [62,63]. Western blots readily detect expression of this early protein by 5 h post infection and it is expressed to high levels late in infection [4,63]. The kinase is packaged in virions as a tegument protein [64], and is posttranslationally modified by phosphorylation, which is apparent in the altered electrophoretic mobility of the protein [4]. Its phosphorylation occurs in the absence of other viral proteins [4], and is consistent with the autophosphorylation observed with the purified enzyme [28]. Intracellular localisation of pUL97 is predominantly nuclear, although some perinuclear staining is apparent very late in infection [4,63].

The UL97 kinase has a large amino terminal regulatory domain that shares some identity with both chimpanzee CMV and rhesus CMV (Figure 1). This domain is not required for enzymatic activity since the deletion of the first 292 aa only reduces phosphorylation of GCV to levels observed in drug resistant mutants with common mutations such as M460V and H520Q [65]. However, important functions have been mapped to this domain including nuclear localisation signals that map between aa 48 and 110 [65]; their utilisation may be affected by its enzymatic activity since null mutants exhibit altered nuclear localisation in transfected cells [66]. The regulatory domain also contains a conserved retinoblastoma (Rb) binding domain [67,68], and its disruption affects the inactivation of the tumour suppressor in infected cells [68], and modestly impacts its susceptibility to MBV [69].

The carboxyl terminal kinase domain of the protein shares greater identity with other protein kinases [8,62], including conserved domains presumed to be involved in catalysis [70] (Figure 1). Consistent with these analyses, mutations within these domains (G340, A442, L446 and F523) reduce its autophosphorylation in recombinant vaccinia viruses [65,71], as well as its ability to phosphorylate GCV [72]. Mutations that confer resistance to GCV also cluster in the conserved domains and confirm that they are involved in phosphorylation [73,74]. Disruption of the invariant lysine residue in conserved region II by K355M or K355Q substitutions also eliminates enzymatic activity and confirms its essential role in catalysis [28,75]. The conserved architecture of this domain facilitated the modeling of the

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three dimensional structure of the catalytic portion of the kinase and predicts that it shares structural features with other kinases [23,73]. However, solving the crystal structure of this enzyme is essential and will be a critical step that will drive the development of additional inhibitors.

Enzymatic Activity and Phosphorylation of Antiviral Drugs

Phosphorylation of protein substrates appears to be the natural function of the kinase and the purified enzyme exhibits autophosphorylation activity on serines and threonines [28]. Optimal autophosphorylation occurs at pH 9.5, 1.5 mM NaCl, requires divalent cations with a preference for Mn^{2+} over Mg^{2+}, and utilises either ATP or GTP as phosphate donors with K_ms of 2 and 4 μM, respectively [28]. Phosphorylation of serine residues in certain peptide substrates indicated that lysine or arginine residues 5 aa downstream of the serine might contribute to the specificity of the kinase [53], but it is not required and a consensus site for the kinase has been very difficult to define [46]. Rather, the specificity of the kinase is likely influenced by proximity to protein substrates and may be mediated in part by physical interactions as has been observed with other kinases [76,77].

The phosphorylation of synthetic nucleoside analogs by the UL97 kinase remains an important focus of research. Initial studies with drug resistant mutants showed that the open reading frame is required for the phosphorylation of GCV [20,78], and that extracts containing the kinase can phosphorylate the drug [19]. This activity is unusual for a ser/thr protein kinase, particularly since it does not appear to phosphorylate natural deoxynucleosides [63]. The purified enzyme also efficiently phosphorylates GCV [28], and acyclovir, albeit to a much lesser extent [79]. Genetic studies suggest that it can also phosphorylate the guanosine analogs penciclovir [80], and cyclopropavir [81].

The activation of antiviral drugs by the UL97 kinase is a critical function that is not generally shared with other homologs, although GCV can be phosphorylated to a limited extent by the EBV BGLF4 kinase [82], and the R97 kinase in rat cytomegalovirus [23]. Neither VZV ORF 47 kinase [83], nor the HSV UL13 kinase appear to phosphorylate the drug to a significant degree [23]. Although MCMV is susceptible to GCV, it does not appear to be mediated by the M97 kinase since deletion of the gene does not impact susceptibility [56], and polymorphisms associated with resistance do not map to the predicted regions of the kinase [74,84]. Chimeric viruses expressing the UL97 kinase have been constructed and might be used to evaluate the efficacy of antiviral drugs in animal models, but efforts to date have met with limited success. While MCMV is a good model to study many aspects of HCMV biology [85], it has not proven to be a useful tool to study the most important inhibitors of HCMV replication used in the clinic [86,87]. A recombinant MCMV expressing the HCMV UL97 kinase is only modestly more susceptible to GCV [56], and the poor complementation of the phenotype suggests that MBV would also be inactive in this system. The expression of the UL97 kinase in guinea pig cytomegalovirus did appear to confer susceptibility to both GCV and MBV and might be used to develop a more useful model to evaluate these drugs [58].

Natural Substrates of UL97 Kinsase

The Ul97 kinase phosphorylates a number of viral and cellular proteins that are considered to be the natural substrates of the kinase (Figure 2, Table 1). The kinase is itself a substrate and autophosphorylates serines 2, 3, 11, 13, 133 as well as threonines 16, 18, 134 and 177 [88]. Deletion of the first 239 aa abrogates autophosphorylation, yet only reduces the phosphorylation of H2B or GCV and confirmed that the phosphorylation of this domain is not required for kinase activity [88]. This is consistent with previous studies showing that the amino terminus is not strictly required for kinase activity [65]. Other HCMV proteins are

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phosphorylated by the kinase, including the ppUL44 DNA polymerase processivity factor, which interacts with, and is directly phosphorylated by, the kinase [37]. The physical interaction of the proteins also occurs in transient expression studies in cell culture and yeast two-hybrid studies suggest that aa 366-459 of pUL97 mediate the binding [38]. Kinase activity is also required for the phosphorylation of ppUL44 in infected cells since it does not occur in the presence of MBV, or in cells infected with RCΔ97 [37]. The pp65 tegument protein is also phosphorylated directly by the purified enzyme and the two proteins interact physically [36], which is consistent with observed colocalisation of the proteins when they are transiently expressed [66]. Consequences of pp65 phosphorylation are not well understood, but infected cells produce large nuclear aggregates containing substantial quantities of this protein in the absence of kinase activity [66,73]. Although these viral proteins appear to be the major viral substrates, other viral gene products that are phosphorylated by the kinase will likely be identified.

Several cellular proteins also appear to be natural substrates of the UL97 kinase and many are also targeted by cdk1. *In vitro* kinase assays with purified proteins have shown that the carboxyl-terminal domain of RNA polymerase II is phosphorylated directly by UL97 kinase [46]. Many other kinases phosphorylate this protein, including cdk1 and cdk7, which regulate its activity through the phosphorylation of serines 2 and 5 of the heptad repeat [49,50]. Roscovitine, an inhibitor of cellular cdks, can also inhibit the phosphorylation of this domain if it is added very early in infection and results in defective immediate early gene expression [89], as well as a failure of cdk9 and cdk7 to accumulate at sites associated with immediate early transcription [90]. However, because roscovitine inhibits several kinases and affects other pathways the effects of the drug on UL97 kinase remain unclear. EF-1 is a substrate of UL97 kinase and is thought to promote the translation of viral messages [44]. The phosphorylation of EF-1 has been best described in HSV, where UL13 and cdk1 can both phosphorylate EF-1 directly [31], and share a similar specificity that results in the phosphorylation of serine 133 [7]. Phosphorylation of EF-1 by the UL97 kinase is thought to perform a similar function and mimics this aspect of cdk1/cyclin B activity, which is thought to regulate EF-1 by phosphorylation [91]. The UL97 kinase is also required during nuclear egress and Marschall and colleagues showed that it interacts with the cellular protein, p32; the complex is then recruited to the lamin B receptor in the nuclear membrane [92]. *In vitro* kinase assays on immunoprecipitated materials suggested that the interaction induces the phosphorylation of both p32, as well as lamins A and C. Lamin A was also shown to be phosphorylated directly by the UL97 kinase on serines that are also phosphorylated by cdk1 [93], and likely directs its redistribution by a similar mechanism [94].

Another important target appears to be Rb, which is normally hyperphosphorylated in infected cells [95] (Figure 3). This phosphorylative inactivation of Rb requires the UL97 kinase and involves both kinase activity as well as a conserved Rb binding domain in the amino terminus of the protein [68]. The kinase appears to phosphorylate Rb directly, and no other proteins appear to be required [67]. Thus, the kinase performs some of the same functions as cellular cyclin-cyclin-dependent complexes, consistent with reports suggesting that the viral kinases mimic cdc2/cdk1 [7,44] (Figure 2). This result is also consistent with results showing that the inhibition of (cdk1/cdk2/cdk5/cdk9) with indirubin-3′-monoxime potentiates the effect of MBV, which suggests that these cellular kinases complement the function of the kinase to some degree [96]. Additional cellular targets will likely be indentified and may reveal additional facets of its activity in infected cells.

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Function in HCMV Replication

The unique characteristics of the UL97 kinase suggest that its function in viral replication will likely be distinct from that of other herpesvirus kinases. Several early reports speculated that the kinase may fulfill an essential function in the virus [4,28,63,65,97], and although severely impaired replication is indeed observed in the absence of the kinase, it is clearly dispensable for viral replication [25]. In cells infected with RCΔ97, progeny virus titers are reduced by more than 100-fold in single step growth curves and more profound impairments occur at lower multiplicities of infection [25,98]. This result is entirely consistent with the potent antiviral activity of MBV and confirms that the kinase is a good target for antiviral chemotherapy [99]. Recombinant viruses with K355M and K355Q mutations also exhibit the same growth defects [67–69]. Studies with null mutants in conjunction with those utilising MBV have been exceedingly useful in understanding the function of the kinase in viral replication and provide an independent means of confirming specific observations. But since the kinase phosphorylates many proteins, it necessarily impacts many viral and cellular processes making the kinase null phenotype both complex and rather cell specific [100].

The activities of the UL97 kinase described above act in concert to support viral replication and each of the specific defects that occur in the absence of its enzymatic activity likely contribute to the poor production of progeny virus (Figure 2). In cells infected with RCΔ97, immediate early and early viral proteins accumulate normally, but a modest reduction in viral DNA accumulation is frequently observed [98,101]. Similar defects in DNA accumulation occur in cells infected with the wt virus in the presence of MBV and confirm that kinase activity promotes DNA synthesis, although the drug does not inhibit the DNA polymerase directly [99]. This defect is likely related to the inactivation of Rb by the kinase, which should stimulate the expression of cellular proteins involved in this process and its impact on DNA synthesis would likely vary depending on the state of the host cells [67,68]. Decreased expression of late viral proteins is observed and may simply reflect modest impairments in viral DNA synthesis [98,101], but may also be the result of more specific defects similar to those described in HSV in the absence of UL13 activity [31,44,48,102].

Deletion of the kinase does not appear to affect the cleavage of concatameric viral DNA [101,103], and is consistent with results observed with MBV in reversal experiments with a specific inhibitor of concatamer cleavage [99]. Effects on encapsidation are less clear. While one study observed reduced encapsidation of unit length viral genomes in DNase protection studies and an over representation of immature capsids [98], a second did not observe any impairments in encapsidation using similar methods [101]. Variable effects on encapsidation may also be related to the state of the host cells [100], as well as the formation of nuclear aggresomes in the absence of UL97 kinase activity which complicates the interpretation of the required assays [68]. Neither report observed the egress of mature capsids from infected nuclei suggesting that replication defects occurred predominantly in the nucleus [101,103]. Inefficient encapsidation [98], impaired morphogenesis [66,68], and failure to induce the degradation of the nuclear lamina all likely contribute to the poor replication phenotype [92].

The formation of mature virions appears to be inefficient in the absence of UL97 kinase activity. Large refractile aggregates are induced in cells infected with RCΔ97 [25], viruses with point mutations in *UL97* that eliminate enzymatic activity [68], or in cells infected with the wt virus when the kinase is inhibited with MBV [66,73]. These structures contain an abundance of viral structural proteins, particularly the pp65 tegument protein and can be reproduced in a transient system in which the aggregation of a pp65-GFP fusion protein can be inhibited by the kinase [66]. Observed aggregates are aggresomes, which are cellular strucures that sequester considerable quantities of viral proteins and their formation was

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specifically inhibited by the kinase [68]. This activity of the kinase is presumed to promote the formation of infectious virus in HCMV through the inactivation of this innate immune response and appears to be similar to the inhibition of autophagy by vMIA [104]. This differs from results in other viruses that have been reported to utilise aggresomes and autophagosomes as sites of viral replication [105,106]. Aggresome suppression appears to be important in HCMV cells since the majority of structural proteins are sequestered in the absence of kinase activity, rendering them unavailable for the production of virus particles.

UL97 kinase activity is required for the redistribution of nuclear lamins and is an important activity since herpesvirus capsids are too large to penetrate the meshwork of nuclear lamins during egress from the nucleus [107,108]. In MCMV, M50/p35 recruits cellular protein kinase C to phosphorylate and disperse the nuclear lamins [109], and similar events occur in HCMV since pUL50 recruits pUL53 and protein kinase C to the nuclear lamina [110]. However, UL97 kinase also appears to be required for this process since UL97 null mutants fail to induce the redistribution of the nuclear lamina [92], as does the wt virus in the presence of MBV [93]. Other important events such as capsid maturation, acquisition of tegument, and transit to the nuclear membrane may also be affected by the kinase, but they remain ill defined in HCMV [111].

HCMV induced structural changes in the cytoplasm also fail to occur normally in the absence of UL97 kinase activity, but their impact on viral replication is unclear. HCMV infection results in the reorganisation of Golgi-related structures near the microtubule organising centre and are thought to be a site of viral assembly [112,113]. This remodelling fails to occur in RCΔ97, or with wt virus in the presence of the kinase inhibitor, NGIC-1, and may also be related to the formation of kidney shaped nuclei in infected cells [93,114]. Specific molecular events resulting in these structural changes remain to be described.

HCMV alters events in the cell cycle to facilitate viral replication [115–117], and requires the expression of early genes [118]. Cellular DNA synthesis and chromosomal segregation are blocked in infected cells, despite the presence of cellular proteins associated with these processes [95,119–122]. The activity of the UL97 kinase appears to be one of the key viral proteins responsible for these changes. Replication of RCΔ97 is highly dependent upon the condition of the host cells, and plaques do not progress in confluent human foreskin fibroblast cells unless they are passaged to induce cell division [25]. Studies with indirubin-3′-monoxime showed that (cdk1/cdk2/cdk5/cdk9) can complement some activities of the UL97 kinase and suggests that they impact similar pathways [96] (Figure 3). Indeed, the UL97 kinase activity is required for the hyperphosphorylation of Rb that normally occurs in infected cells [68]. Recombinant viruses with mutations in either the essential lysine (K355M), or the consensus Rb binding domain in the amino terminus of the kinase (C151G) exhibited reduced phosphorylation of serine 780 [68], which inactivates Rb and renders it unable to interact with E2F [123,124] (Figure 3). The inactivation of Rb by the UL97 kinase is mediated by direct phosphorylation and its expression in recombinant adenoviruses stimulates the cell cycle, confirming that it can stimulate the cell cycle [67]. Additional consequences of Rb inactivation by the kinase in viral replication remain to be described, but its impact on the cell cycle likely reflects shared functions with cyclincyclin-dependent kinases. This activity is important because critical events in the cell are regulated by Rb, and its phosporylative inactivation by the UL97 kinase is reminiscent of the activity of viral oncogenes such as adenovirus E1A, papillomavirus E7 and SV40 large T antigen. It is therefore conceivable that the UL97 kinase may have certain oncogenic properties and is a hypothesis that must be seriously entertained. If such a proliferative disorder were identified, it is certainly possible that inhibition of UL97 kinase activity by maribavir might impact the clinical course of the disease.

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UL97 Kinase as a Target for Antiviral Therapy

Additional therapies are clearly required to treat HCMV infections [125–127], particularly those that are underserved by existing drugs [128,129]. Critical functions performed by the kinase make it an excellent target for the development of antiviral drugs and inhibitors of its activity represent a new class of antiviral drugs [130–132]. A number of inhibitors have been described and include indolocarbazoles [75,82,133], quinazolines [134], as well as benzimidazole ribosides [99]. MBV is a benzimidazole L riboside and is the only highly specific inhibitor of the UL97 kinase [135,136]. This drug is currently under clinical development and the literature has been reviewed recently [137]. It exhibits favourable pharmacokinetic properties, is well tolerated and holds promise as a new drug for the treatment of HCMV infections [138–140],

Inhibition of UL97 kinase activity by MBV was established early in the development of the drug [99], and its complex mechanism of action corresponds to the UL97 null phenotype described above (Figure 2). Studies with the drug have contributed significantly to our understanding of UL97 function and recently have been expertly reviewed [73]. Drug resistant mutants can be selected in the laboratory, and arise more frequently in strains of the virus with mutations in the exonuclease domain II of the DNA polymerase [141]. Mutations that arise are distinct from those of GCV resistant mutants and some lie outside the conserved kinase domains (Figure 1) [73,142,143]. Consistent with these observations, the drug remains active against GCV resistant strains and should be useful in the treatment of drug resistant infections [73,99,144]. However, the inhibition of UL97 kinase activity by MBV may interfere with the activation of GCV if administered concomitantly [145], and it has been reported to occur in cell culture [146]. Clinical studies need to be designed with this issue in mind, but strategies to minimise this effect clearly exist.

Interestingly, most resistant strains isolated in the laboratory do not have mutations in *UL97*, but rather map to the *UL27* open reading frame [147,148]. The resistance conferred by mutations within *UL27* is modest compared to those in the kinase, but they appear to occur much more frequently. This protein has no reported function, but the deletion of *UL27* results in a modest half log reduction in viral replication *in vitro*, and no apparent effect on replication *in vivo* [149]. It is unclear how pUL27 impacts the activity of MBV, but it is possible that it modulates pUL97 activity by some mechanism and will elucidate an important new aspect of the UL97 phenotype.

Concluding Remarks

Parallels between the activities of UL97 kinase and cdc2/cdk1 are striking and appear to represent a thread common to all the herpesvirus kinases [7]. While functions of the UL97 kinase in cell culture are comparatively well understood, the impact of these activities on infected individuals is unknown. It is possible that the cell cycle stimulation resulting from its inactivation of Rb may drive cell proliferation and contribute to conditions not yet attributed to viral infection. Clinical trials with MBV will be critical in advancing our understanding of UL97 function during human infection, and may identify conditions that respond to therapy and related to the activity of the kinase. More importantly, it will demonstrate the efficacy of a new therapeutic strategy that can be applied to the development of antiviral therapies for other viral infections.

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Abbreviations

cdk1/cdc2	cyclin-dependent kinase 1
EF-1	elongation factor 1delta
GCV	ganciclovir
MBV	maribavir
MCMV	murine cytomegalovirus
Rb	retinoblastoma protein

Figure 1.

Conserved domains in pUL97. The aa sequence for pUL97 (CAA35333.1) is shown with conserved kinase domains highlighted by gray blocks [70]. Amino terminal domains conserved among the primate cytomegalovirus *UL97* homologs (HCMV (CAA35333.1), rhesus cytomegalovirus (AAC05259.1), and chimpanzee cytomegalovirus (NP_612729.1) are highlighted by yellow blocks. Sequences required for nuclear localisation signals are underlined in green (aa 48-110) [65], and the interaction domain with ppUL44 between aa 365-459 [38]. Phosphorylated serine and threonines are shown in red with asterisks below [88]. GCV resistance is associated with mutations in blue aa with deletions occurring in the area underlined in blue [73,74]. Mutations that confer resistance to MBV are shown in boxed red text [73]. Rb binding motifs are underlined bold text [67,68], and the invariant lysine 355 is boxed in black

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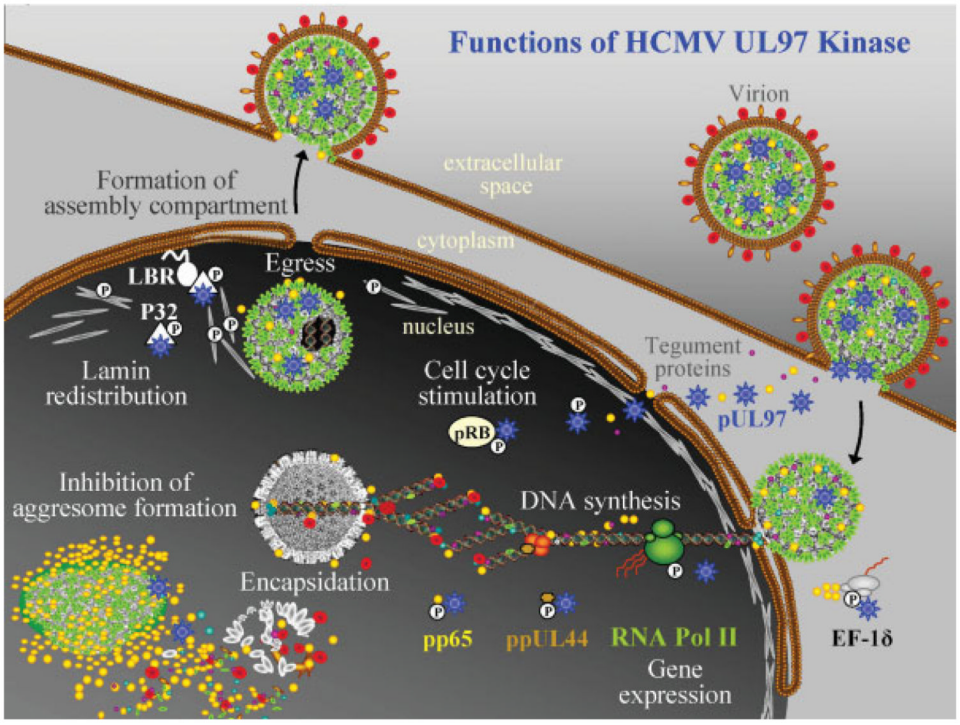

Figure 2.

Function of UL97 kinase in viral infection. Infection with HCMV results in the fusion of the virus particle with the cell membrane and the release of tegument proteins including pUL97 (shown as blue stars) into the host cell. This enzyme is also expressed early in infection and directs the phosphorylation of the viral proteins, ppUL44 and pp65, as well as cellular proteins. Phosphorylation of Rb results in the stimulation of the cell cycle and promotes the synthesis of cellular enzymes that help facilitate HCMV DNA replication. Phosphorylation of the RNA polymerase II large subunit as well as EF-1 is also thought to promote the expression of viral genes. The kinase also contributes to virion morphogenesis by inhibiting the formation of nuclear aggresomes, which sequester considerable quantities of viral structural proteins in the absence of its activity. The kinase then directs the phosphorylation and redistribution of the nuclear lamins to facilitate the egress of mature virions from the nucleus. The kinase is also thought to affect the formation of the assembly compartment in the cytoplasm. Inhibition of UL97 kinase activity by maribavir results in the accumulation of aggresomes and immature virions in the nucleus which are unable to pass through the nuclear cage; thus, very little infectious virus is produced or released from the cell. Although the kinase is delivered to the cell as a tegument protein, the kinase expressed during the infection appears to mediate most effects

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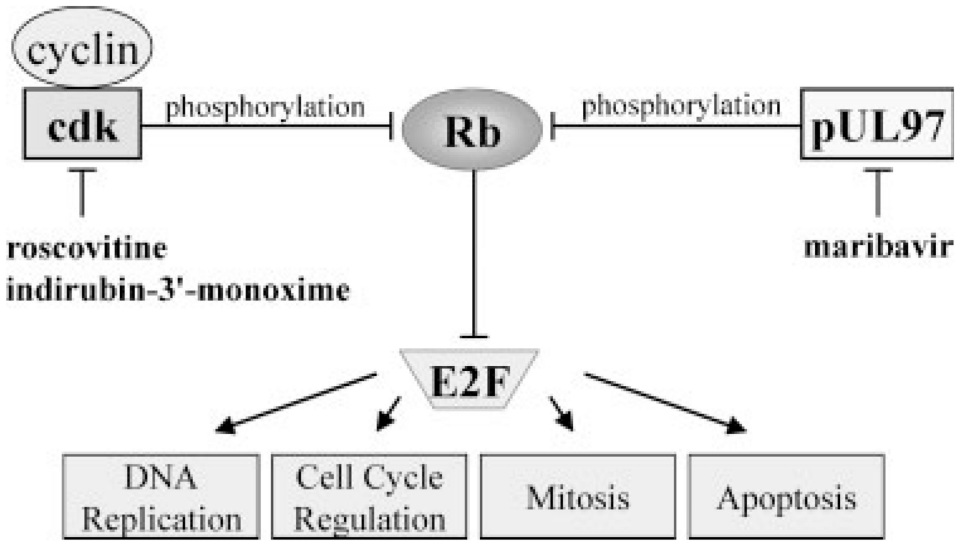

Figure 3.

Inactivation of retinoblastoma protein (Rb) by UL97 kinase and its impact on the cell. In quiescent cells, Rb binds and inhibits the activity of the transcription factor, E2F. During the cell cycle, Rb is phosphorylated by cyclin-dependent kinases (cdk) complexed with cyclins. This phosphorylation inactivates Rb, and leads to the release of active E2F, which promotes the transcription of factors involved in DNA synthesis, cell cycle regulation, mitosis and apoptosis. In infected cells, the UL97 kinase performs a similar function and also inactivates Rb, leading to the release of active E2F. Compounds, such as roscovitine, inhibit the enzymatic activity of cellular cdks and thus prevent the inactivation of Rb. Maribavir is a specific inhibitor of UL97 kinase and inhibits the inactivation of Rb only in infected cells

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Table 1

Natural substrates of the UL97 kinase

Substrate	Putative function	Evidence	References
ppUL44 DNA polymerase processivity factor	Viral DNA synthesis	Direct phosphorylation	[37,38]
pp65 tegument protein	Morphogenesis, assembly	Direct phosphorylation	[36,150]
RNA polymerase carboxy terminal domain	Immediate early gene expression	Direct phosphorylation	[46,89,90]
Eukaryotic elongation factor 1delta	Activation of protein Synthesis	Direct phosphoryation	[31,44]
Retinoblastoma protein	Modulation of cell cycle	Direct phosphoryation	[67,68,95]
Lamins A and C, p32	Process of nuclear egress	Direct phosphorylation; phosphorylation of immunoprecipitated material	[92,93,98,101,110]