The Human Cytomegalovirus UL112-113 Locus Can Activate the Full Kaposi’s Sarcoma-Associated Herpesvirus Lytic Replication Cycle

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Human cytomegalovirus (HCMV) infection of a cell containing latent Kaposi’s sarcoma-associated herpesvirus (KSHV) results in the activation of KSHV lytic replication and the production of infectious virus. In this study, we investigated the HCMV genes identified as having a role in the activation of HCMV early genes for their ability to activate KSHV lytic replication. It was found that the UL112-113 locus was able to activate the complete KSHV lytic cycle, while the UL122-123 locus, encoding the IE1 and IE2 proteins, known to be strong transactivators, did not.

Kaposi's sarcoma-associated herpesvirus (KSHV), or human herpesvirus 8, is a gamma herpesvirus involved in three neoplastic diseases: Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease (23, 28). These diseases are most often found in persons with impaired immune responses, as evidenced by that fact that KSHV causes significant disease in people who are immunosuppressed due to human immunodeficiency virus infection or iatrogenic causes (2–4, 10). Individuals at increased risk for KSHV-related diseases due to immunosuppression often have other exacerbated ongoing infections, and there is the possibility of interactions between pathogens that could impact KSHV pathogenesis, as suggested by reports of the activation of KSHV lytic replication by HIV (15, 35), human cytomegalovirus (HCMV) (36), herpes simplex virus (26, 36), and human herpesvirus 6 (19). As with all herpesviruses, KSHV alternates between lytic and latent states of replication, which allows the lifelong infection of a host. The reactivation of herpesvirus from latent to lytic replication plays a critical role in the transmission of the virus between hosts, the dissemination within a host, and the pathogenesis of these viruses. HCMV is a common pathogen, with generally more than 50% of people being infected, and active HCMV infection is common in people who are immunosuppressed (25). KSHV and HCMV can infect many of the same cell types, and we previously found that KSHV lytic replication can be activated by coinfection with HCMV (36). The mechanism of KSHV activation by HCMV is unknown, and investigation of the process may give a better understanding of the switch from latent to lytic replication for KSHV and the function of HCMV genes. The first step in understanding the process by which HCMV activates KSHV lytic replication is the identification of the HCMV gene(s) responsible, and in this study, we investigated the capability of HCMV loci involved in the transactivation of HCMV genes to activate KSHV lytic replication.

KSHV and HCMV can infect many of the same cells, and the activation of KSHV lytic gene expression by HCMV in a number of these cell types, including human fibroblasts (HF), human umbilical cord endothelial cells, and keratinocytes, has been demonstrated (36). HCMV was also found to activate KSHV lytic gene expression in cells not permissive for the complete replicative cycle of HCMV. This was examined in Vero cells infected with rKSHV.219, a recombinant virus that expresses the green fluorescent protein (GFP) in essentially all infected cells from the cellular EF-1a promoter and expresses the red fluorescent protein (RFP) only in cells with KSHV lytic gene expression from the lytic PAN promoter. The PAN promoter is directly activated by RTA (30), the major activator of KSHV lytic gene expression (21, 34). Figure 1 shows results of fluorescent microscopy used to detect GFP and RFP in Vero cells that contained rKSHV.219 and were either mock infected or infected with HCMV (Towne) at a multiplicity of infection of 5 (as determined by virus titers on HF). Although Vero cells are not efficiently infected by HCMV (Towne), the expression of RFP is indicative of lytic gene expression activated by HCMV coinfection. Because HCMV could activate KSHV lytic gene expression in Vero cells, they were frequently used for transfection experiments described below for their higher transfection efficiency, and greater production of KSHV, than HF.

With the hypothesis that the HCMV genes responsible for activating HCMV early viral genes (16, 24) are the genes involved in activating KSHV, these HCMV genes were tested for their ability to activate KSHV. This set of HCMV genes includes the UL122-123 locus, encoding the IE2 (p86) and IE1 (p72) proteins, which are the major transactivators of HCMV viral genes (31, 33), and are also capable of promiscuous activation of other promoters (17). TRS1, and the closely related IRS1, are two genes constituted in part by repeated sequences flanking the unique short domain, and by unique short sequences, such that the amino-terminal ends are homologous while the carboxy-terminal ends diverge. Functions of the TRS/IRS genes include increasing gene expression and binding double-stranded RNA (14, 16, 32). The UL112-113 locus encodes four phosphorylated nuclear proteins, which are associated with sites of viral replication and have been found to have a role in the activation of some early genes (1, 16, 42, 43). The
UL36-38 locus was shown to cooperate with other viral proteins for the activation of viral and cellular promoters (8).

The ability of these four HCMV loci to activate KSHV lytic replication was tested by the transfection of the plasmids pON303G (UL122-123) (7), pON2334 (TRS) (32), pZP8 (UL36-38) (24), and pZP24 (UL112-113) (16) in different combinations into HF or Vero cells containing latent rKSHV.219. The transfections were carried out using 5 μg of plasmid by electroporation (voltage, 320 V; capacitance, 1,200 μF; resistance, 3 Ω) with cells suspended in RPMI–10% fetal bovine serum using a BTX ECM600. A plasmid expressing the cerulean fluorescent protein (DNA 2.0, Menlo Park, CA) expressed by a murine CMV enhancer/elongation factor 1α promoter (Invivogen, San Diego, CA) was used as a control for transfection efficiency in all electroporations. The HCMV genes in the plasmids used were all expressed by their viral promoters, so the TRS, UL112-113, and UL36-38 genes would have low, if any, expression if transfected alone, because these genes require activation by HCMV-expressed proteins, such as the IE1 and IE2 proteins encoded by UL122-123, for full expression. Therefore, the transfection strategy for this experiment included testing the ability of TRS, UL112-113, and UL36-38 to activate KSHV lytic gene expression with cotransfection of the UL122-123 locus, as well as transfection without UL122-123. The transfected cultures were scored for the production of infectious KSHV by infecting 293 cells with cell-free supernatant from the cultures 3 days posttransfection and counting GFP-positive 293 cells 2 days postinfection, as previously described (37). The results of these transfections are presented in Fig. 2 and demonstrated that the combination of UL122-123 and UL112-113 was sufficient to activate the production of infectious KSHV in both HF and Vero cells. Although not able to activate KSHV, TRS and UL36-38 did contribute an additive effect. It was also notable that the UL122-123 locus was not able to activate KSHV lytic replication alone, indicating that the activation by HCMV was not due simply to the strong transactivating functions of the encoded IE1 and IE2 proteins of this locus.

Because the UL112-113 locus was expressed from its viral promoter, it would require IE72 and IE86 for maximal expres-

![FIG. 1. Vero cells containing latent rKSHV.219, with mock infection (A and B) and HCMV (Towne) infection at a multiplicity of infection of 5 (C and D), imaged for GFP (A and C) or RFP (B and D).](image)

FIG. 2. Transfections of HCMV genes into HF and Vero cells containing rKSHV.219. (A) Levels of infectious rKSHV.219 present in cell-free supernatant from Vero/rKSHV.219 and HF/rKSHV.219 cultures transfected with the following HCMV genes: lane 1, UL122-123; lane 2, TRS, UL112-113, and UL36-38; lane 3, UL122-123, TRS, UL36-38, and UL112-113; lane 4, UL122-123, TRS, and UL36-38; lane 5, UL122-123, TRS, and UL112-113; lane 6, UL122-123, UL36-38, and UL112-113; lane 7, UL122-123 and TRS; lane 8, UL122-123 and UL112-113. Values are averages from two experiments for both cell types. (B) Representative photomicrographs imaged with fluorescence for GFP of 293 cells inoculated with cell-free supernatant from the transfected Vero/rKSHV.219 cultures. The transfection number, as shown in panel A, is listed below each panel, with GFP-positive cells indicating infection by rKSHV.219. Inoculation with the supernatants from the other transfections resulted in no GFP-positive 293 cells.
sion, so it could not be determined from the first transfection experiment whether the UL122-123 locus had a role in activating KSHV or was simply necessary to activate the expression of UL112-113 expression or, conversely, if the UL122-113 locus could activate KSHV alone. To test whether constitutive high-level expression of UL112-113 could activate KSHV lytic replication, the UL112-113 locus, nucleotides 160581 to 162800 (AD169 sequence [6]), was cloned by PCR and placed under the control of the HCMV immediate early (IE) promoter to create pQ220. pQ220 was used to transfect cells latently infected with rKSHV.219, which were then compared to cells transfected with the UL122-123 locus or the KSHV RTA gene expressed by the HCMV IE promoter (37), and the transfected cultures were examined for RFP expression and the production of infectious KSHV. The examination of the cultures for RFP expression demonstrated the activation of lytic gene expression by UL112-113, which was much greater than UL122-123 activation, although much less than RTA activation (Fig. 3A). The results from the determination of infectious virus mirrored that of the RFP expression and showed that UL112-113 was sufficient to activate the full productive lytic replication of KSHV, while for the UL122-123-transfected cells, no infectious virus was detected (Fig. 3B).

The lytic replication of a herpesvirus begins with the expression of the immediate genes that activate the early genes, which carry out viral DNA replication, resulting in the expression of late genes and virus production. The activation of KSHV by HCMV could proceed by the activation of the KSHV major IE gene, RTA, or HCMV could directly activate the E genes and perhaps bypass the need for RTA. Therefore, we next tested the ability of HCMV to activate the RTA promoter, as well as one early promoter, the ORF6 promoter. The activity of the RTA promoter was determined by using the RTA promoter (nucleotides 70693 to 72598 [27]) to express the secreted alkaline phosphatase and assayed as described previously (5). The ORF6 promoter (nucleotides 2769 to 3208 [27]) was used to express the lacZ gene, and its activity level was determined using a 4-methylumbelliferyl-4-D-galactoside cleavage assay (12). To examine the activation of these two promoters by HCMV, HF were electroporated with the RTA/secreted alkaline phosphatase or ORF6/lacZ construct, and transfected cells were evenly split between wells that were infected with HCMV and wells that were mock infected. The analysis of promoter function demonstrated that HCMV could activate the early promoter 4-fold and the IE promoter more than 100-fold (Fig. 4, left). These results indicate that the activation of KSHV lytic replication by HCMV proceeds by the activation of RTA expression but does not rule out the possibility that the activation of early genes could be an augmenting activity for the production of virus.

Because HCMV was able to activate the RTA promoter, and it was found that the UL112-113 locus was able to activate the production of infectious KSHV, the UL112-113 locus was tested for its ability to activate the RTA promoter and the ORF6 promoter (Fig. 4, right). These transfection experiments demonstrated that the UL112-113 locus was capable of directly activating the RTA promoter, in keeping with its ability to activate lytic replication, but could not activate the ORF6 promoter. Although the activation of the RTA promoter by UL112-113 is modest, because the RTA protein can autoactivate its own expression (9), it may be that the role of the UL112-113 locus in activating KSHV is to turn on the expres-
sion of RTA, and then the autoactivation of its own expression achieves the levels of expression necessary for full activation of lytic replication.

These experiments showed that the HCMV UL112-113 locus is capable of activating the full lytic replication cycle of KSHV with the production of infectious virus. The UL112-113 locus is capable of activating the KSHV RTA gene promoter, which is the KSHV gene responsible for initiating KSHV lytic gene expression. The UL112-113 locus encodes four phosphorylated proteins, pp84, pp50, pp43, and pp34 (38, 41, 42). The UL112-113 proteins have been localized to sites of HCMV DNA replication associated with promyelocytic leukemia protein-associate nuclear bodies (1). Although the UL112-113 locus is not absolutely necessary for HCMV replication, the loss of its functions results in a virus with severely reduced replication (11). A function of the UL112-113 proteins in promoter transactivation has been described for the HCMV UL54 promoter both in association with the IE2 protein (18) and, to a lower level of activation, alone (43). In transient-transfection assays, the activation of the UL54 promoter by HCMV proteins involved the binding of cellular proteins to an inverted repeat in the promoter (18). In contrast to the UL54 promoter, which was activated by the IE proteins to a higher level than by the UL112-113 proteins, the KSHV RTA promoter was activated only by UL112-113. It is not known if the activation of the RTA promoter by UL112-113 is mediated through cellular proteins, but this is a possibility, since a variety of cellular proteins have been implicated in RTA activation, including API (39, 44), XBP-1s (40, 46, 47), and Sp1 (45), as well as Ets-1 and other transcription factors (47). It is also possible that the UL112-113 proteins exert their affect through chromatin-related mechanisms, as the roles reported for sodium butyrate and valproic acid (histone deacetylase inhibitors) and for chromatin remodeling proteins in the activation of KSHV (13, 20, 22, 29, 41). The role of cellular proteins, the possibility of the UL112-113 proteins interacting directly with the RTA promoter, and any differential role for the four proteins encoded by the UL112-113 locus still need to be investigated.

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REFERENCES


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