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Viral Gene Products Actively Promote Latent Infection by Epigenetic Silencing Mechanisms

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Abstract

Many viruses undergo an acute infection in the host organism and then are cleared by the ensuing host immune response, but other viruses establish a persistent infection involving a latent infection or a chronic infection. Latent infection by the herpesviruses or human immunodeficiency virus involves epigenetic silencing of the DNA genome or proviral genome, respectively. Latent infection was previously thought to be a default pathway resulting from infection of a nonpermissive cell, but recent studies have shown that viral gene products can promote epigenetic silencing and latent infection. This review will summarize the viral gene products that have been shown to promote epigenetic silencing of their genomes and the potential for therapeutics to target these viral gene products and disrupt or lock in latent infection.

Introduction

Many viruses undergo an acute infection in the host organism and then are cleared by the ensuing host immune response. However, other viruses establish a persistent infection by either establishing a latent infection, in which the virus is quiescent and no infectious virus can be detected, or a chronic infection, in which infectious virus is continually produced. The herpesviruses are good examples of viruses that establish a latent infection. Herpes simplex virus (HSV) undergoes an acute lytic infection in the mucosal epithelium and spreads to establish a latent infection in sensory neurons. Historically, there was debate about whether HSV latent infection was truly quiescent or “static” versus slowly replicating or “dynamic” [1]. The studies of Jack Stevens establishing a murine model of latent HSV infection showed that the virus is quiescent during latent infection in this murine infection model [2]. There is little to no oral shedding of HSV-1 from humans, but Corey and colleagues have detected frequent low level HSV-2 genital shedding in certain study populations [3], and they have argued for a chronic infection. However, their modeling studies argue that only a low fraction of neurons are reactivating at any time in these individuals [4], so the bulk of HSV-2 latent infection is relatively quiescent. In murine

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models of HSV-1 latent infection, there is low level expression of lytic transcripts [5,6], and this is likely due to abortive or nonproductive reactivation events [5,7].

A second important question concerning viral latent infection has been whether it is a default pathway due simply to viral infection of non-permissive cells or whether the virus plays an active role in the establishment of latent infection. Originally, viruses were thought to undergo a latent infection when they enter a non-permissive cell and go quiescent as a default pathway. Recent studies have shown that several viral gene products can actively promote latent infection by epigenetic silencing of their respective viral DNA genomes, by preventing cell death, or by tethering of the viral genome to cellular chromosomes in dividing cells. In this mini-review, we will focus on those gene products that have been shown to promote epigenetic silencing and thereby facilitate latent infection.

Herpes simplex virus (HSV)

HSV undergoes a lytic infection in epithelial cells and fibroblasts and spreads to establish a latent infection in sensory neurons. In epithelial cells, HSV expresses its lytic gene products in a cascade of immediate-early (IE), early (E), and late (L) gene products. The HSV virion protein 16 (VP16) forms a complex in the infected cell nucleus with the cellular Oct-1 and host cell factor 1 (HCF-1) proteins in which Oct-1 binds to IE gene promoters and HCF-1 recruits epigenetic factors to remove heterochromatin marks and add euchromatic marks to viral chromatin [8]. The IE infected cell protein 4 (ICP4) and ICP0 proteins promote E and L gene transcription. ICP4 recruits RNA polymerase II and Mediator complex to viral promoters [9]. ICP0 is an E3 ubiquitin ligase that promotes the degradation of a number of cellular proteins, a process that leads to the removal of heterochromatin and addition of euchromatin to E and L gene promoters [10,11] and the inhibition of several intrinsic and innate immune mechanisms [12].

Latency-associated transcript

In sensory neurons HCF-1 is localized in the cytoplasm and cannot form the activator complex with VP16 to activate IE gene transcription [13]. In addition, during the establishment and maintenance of latency, a neuron-specific enhancer drives the expression of the latency-associated transcript (LAT), a 10kb primary transcript that is processed into a stable intron and possibly miRNAs [12]; therefore, the LAT transcriptional unit is the major viral gene transcribed during HSV latent infection. LAT was the first viral gene product shown to increase heterochromatin [14] and reduce viral lytic gene expression [15,16] during establishment or maintenance of latent infection. Although one paper reported that LAT decreases heterochromatin [17], all studies that examined mutant and rescued viruses in parallel have shown that LAT expression increases heterochromatin and/or reduces lytic viral gene expression during latent infection in neurons [14,15,18–20]. It was hypothesized that LAT functions as a long noncoding RNA (lncRNA) to recruit Polycomb repressive complex 2 (PRC2) [21] that adds the heterochromatic H3K27 methylation to viral chromatin (Figure 1), but thus far, no effect of LAT on PRC2 complex subunit recruitment to viral lytic promoters has been observed during latency [22].

ICP0

Given our knowledge of the role of ICP0 in lytic infection, we were surprised to find that *ICP0* promoter and nonsense mutant viruses show reduced heterochromatin during latent infection in trigeminal ganglia of mice [23]. This was surprising because it was the opposite effect of ICP0 to that observed in fibroblasts and epithelial cells. Previous studies had shown that the *ICP0* gene promoter is expressed in at least one third of the neurons establishing latent infection [24] and that ICP0 promoted latent infection in neurons *in vitro* [25], but there was no prior evidence that ICP0 promoted epigenetic silencing or latent infection *in vivo*. Expression of LAT was also reduced in the ganglia latently infected with an *ICP0* nonsense mutant virus [23], which led us to hypothesize that the effect of ICP0 on latent chromatin was indirect by increasing LAT expression, which ultimately resulted in enhanced heterochromatin (Figure 1). This argues that ICP0 plays a role in establishment and/or maintenance of latent infection. Furthermore, this model would suggest that ICP0 could be an attractive target for small molecules that could serve as therapeutics for latent infection in addition to lytic infection.

Human cytomegalovirus (HCMV)

HCMV undergoes a productive infection in primary fibroblasts, but establishes a latent infection when it infects hematopoietic progenitor stem cells [26]. In the latter cells, lytic genes are repressed and only a few latency-associated transcripts are expressed. Like other herpesviruses, the HCMV genome is maintained as a chromatinized episome in latency. Similar to HSV-1, HCMV genomes are not silenced by DNA methylation [27]. In CD34⁺ cells, the major immediate-early promoter (MIEP) is repressed through association with Daxx (death domain associated protein 6), histone deacetylases, heterochromatin protein 1 (HP1) and H3K9me3 heterochromatin while the chromatin associated with LUNA (latency unique natural antigen) gene promoter is acetylated during latency [28]. Daxx acts as a repressor by recruitment of histone deacetylases to lytic gene promoters [28a]. During lytic infection, virion protein pp71 targets hDaxx for degradation, thus enabling enhanced acetylation of chromatin associated with lytic promoters, loss of HP1 and lytic gene expression ensues [28]. During latency, tegument pp71 is retained in the cytoplasm, and IE1 transcription is suppressed [29]. At least two HCMV gene products have been shown to promote epigenetic silencing of the viral genome to enable latent infection.

U_L138

Assembly of heterochromatin onto the CMV MIEP depends on the *U_L138* gene product [30,31]. U_L138 inhibits recruitment of two histone demethylases (KDMs) JMJD3 (KDM6B) and LSD1 (KDM1A) that demethylate and reverse heterochromatic H3K27me3 and H3K9me2, respectively [30], thereby preventing activation of the major immediate-early promoter (Figure 1).

Long non-coding RNA (lncRNA) 4.9

LncRNA4.9 has been reported to physically interact with the MIEP and the EZH2 and Suz12 components of PRC2. The MIEP is associated with increased H3K27me3 and decreased H3K4me3, [32], suggesting that lncRNA4.9 promotes heterochromatin and

epigenetic silencing (Figure 1). It will be important to test a mutant virus that does not express lncRNA4.9 to rigorously test this hypothesis.

Epstein-Barr Virus (EBV)

EBV undergoes a productive infection in oral epithelial cells and then spreads to infect B lymphocytes, which results in a series of latency states characterized by different patterns of EBV gene expression. The initial infection of resting B lymphocytes results in a state (latency III) with expression of a number of latent gene products including Epstein-Barr nuclear antigen (EBNA) -1, -2, 3A-C, EBNA-LP (EBNA leader protein), latent membrane protein (LMP) -1, -2A, -2B, and the EBER transcripts [33]. Latency genes are down-regulated to give rise to the latency II phenotype, in which viral gene expression includes EBNA1, LMP-1 and -2A, and the EBERs. The most common latency form observed *in vivo* shows expression of no EBV proteins (latency 0), but dividing memory B cells are also observed *in vivo* that express only EBNA1 (latency I phenotype). The mechanisms that lead to down-regulation of the latent gene products have not been fully elucidated, but several gene products have been shown to contribute to this gene silencing.

EBV BNRF1

The BNRF1 protein is a virion tegument protein that binds to Daxx and promotes viral gene expression. Daxx functions as a histone H3.3 chaperone that, with ATRX (α -thalassemia X-linked mental retardation) protein, assembles H3.3 and repressive heterochromatin on target genes. BNRF1 disrupts the Daxx-ATRX histone chaperone complex and this results in enhancement of both lytic and latent gene expression [34]. Knockdown of Daxx or ATRX disrupted latency [34]. Mutant viruses expressing BNRF1 incapable of binding to Daxx could not initiate expression of latency proteins [35], arguing that BNRF1 disrupts Daxx-ATRX complexes to promote both lytic and latent gene expression (Figure 1). This is strikingly similar to the situation with HSV-1 ICP0 described above, in which ICP0 also inhibits the Daxx-ATRX histone chaperone complex by promoting the degradation of ATRX, resulting in enhancement of both lytic and latent gene transcription.

EBV miRNAs

Several EBV miRNAs inhibit lytic gene expression, thereby indirectly promoting silencing and latent infection (Figure 1). miR-BART20-5p directly targets the viral IE *BRLF1* and *BZLF1* transcripts and the cellular *Bcl-2*-associated death (BAD) promoter. BAD-mediated caspase-3-dependent apoptosis can trigger IE gene expression and lytic cycle induction; therefore, miR-BART20-5p suppression of BAD also indirectly inhibits lytic gene expression to maintain latent infection [36]. miR-BART18-5p targets mitogen-activated protein (MAP) kinase kinase kinase 2 (MAP3K2). MAP3K2 is part of the signaling pathway that induces lytic viral replication, and therefore, miR-BART18-5p promotes the maintenance of latent infection [37]. miR-BART6-5p can suppress EBNA2 expression, as well as Zta and Rta viral proteins that are essential for lytic replication [38].

Latent Membrane Protein 1 (LMP1)

LMP1's C-terminal activating region-3 (CTAR3) interacts with a SUMO-conjugating enzyme, Ubc9, during latent infection to promote sumoylation of cellular proteins. One target is the KRAB-associated protein-1 (KAP1), a tripartite motif family protein associated with transcriptional repression. LMP1-CTAR3-mediated sumoylation of KAP1 promotes KAP1 binding to cellular chromatin and to EBV OriLyt and immediate-early promoters, which can promote transcriptional repression (Figure 1) and maintain latency [39].

Human herpesvirus 8 or Kaposi's sarcoma-associated herpesvirus (KSHV)

KSHV is primarily associated with B cells but is also associated with endothelial cell tumor cells and can enter a broad variety of cells [40]. The default pathway of infection is a latent infection, but spontaneous reactivation is observed in a fraction of the cells. KSHV latent infection is associated with Kaposi's sarcoma, primary effusion lymphoma (PEL), and multicentric Castleman's disease (MCD). Several viral gene products are known to promote epigenetic silencing of the viral genome and latent infection.

Latency-associated nuclear antigen (LANA)

LANA is expressed during latent infection and plays a role in 1) tethering of the viral episome to host chromosomes for propagation during cell division, and 2) promoting epigenetic silencing of the latent viral episome (Figure 1). With regard to epigenetic silencing, LANA can induce sumoylation and relocalization of soluble Sp100 (not localized to Nuclear Domain 10 structures) from the nucleoplasmic and chromatin-associated fraction to the insoluble matrix. Soluble Sp100 acts as a negative regulator of PRC2 recruitment; therefore, LANA-driven relocalization allows accumulation of facultative H3K27me3 on viral heterochromatin across the viral genome to promote latency establishment [41].

Second, LANA recruits nucleosome assembly protein 1-like protein 1 (NAP1L1) to the terminal repeat (TR) region of the viral genome to stimulate LANA dependent-plasmid replication, maintain episomal DNA, promote chromatin assembly on newly synthesized KSHV DNA, and silence lytic viral gene expression [42]. Third, LANA binds the KSHV genome rapidly after infection and recruits PRC complexes to the viral lytic promoters. Recruitment of PRC2 component EZH2 to viral lytic promoters facilitates the deposition of H3K27me3 during *de novo* infection. Deletion of the *LANA* gene abolishes EZH2 recruitment resulting in reduced H3K27me3 and increased lytic gene expression [43]. KSHV also induces Nrf2 activation, increasing total levels and phosphorylation. Early in *de novo* infection Nrf2 binds and activates the ORF50 promoter to allow a burst of lytic gene expression, Nrf2 then recruits LANA and the host transcriptional repressor KAP1, resulting in the repression of the lytic Orf50 promoter and the establishment of latency [44].

Polyadenylated nuclear RNA (PAN RNA)

PAN RNA is an lncRNA found within infectious virions, expressed at early times post infection, and expressed in latent infection in the absence of lytic induction. PAN RNA can interact with specific demethylases (UTX and JMJD3) and recruit these to the viral genome to remove H3K27me3 and mediate activation of viral gene expression in lytic infection [45].

However, PAN RNA can also interact with PRC2 components to target these complexes to cellular genes to alter cell cycle, enhance cell growth and survival, and suppress the immune response and production of inflammatory cytokines. While most viral gene expression is upregulated by PAN RNA, PAN can also interact with PRC2 and suppress the expression of viral genes K15, K12, ORF20, ORF31, ORF53, ORF67A, and ORF75 [45]. ORF75 is the tegument protein that targets ND10 components ATRX/Daxx to promote lytic infection [46]. Thus, PAN inhibition of ORF75 could allow for ND10 repression and promote latency (Figure 1).

K-bZIP protein

K-bZIP is a KSHV SUMO-2/3-specific E3 ligase that has been proposed to increase SUMO2/3 on viral chromatin during reactivation. A K-bZIP SUMO E3 ligase dead mutant virus shows no SUMO2/3 enrichment on viral chromatin, higher expression of viral lytic genes during reactivation, and increased virus production [47], arguing for the role of K-bZIP in genomic silencing.

KSHV microRNAs

miR-K12-9* [48] and miR-K12-7-5p [49] inhibit translation of the viral immediate early gene ORF50/replication transactivation activator (RTA), resulting in reduced expression of RTA and the downstream early genes regulated by RTA, which reduces transactivation of viral lytic genes. Therefore, these microRNAs indirectly promote silencing of viral genes.

Human Immunodeficiency virus

Human immunodeficiency virus (HIV) uses its reverse transcriptase enzyme to copy its genomic RNA into cDNA and then double-stranded DNA within subviral particles in the cytoplasm [50]. The DNA is then transported in this preinitiation complex into the cell nucleus, where the virus-encoded integrase mediates the integration of the viral DNA into cellular chromosomes to give a proviral genome. In activated T lymphocytes the provirus is transcribed by the cellular RNA polymerase II to give viral transcripts, but in resting T cells the provirus is silenced to establish a latent infection. Latent proviruses are silenced by heterochromatin containing H3K27me3 and H3K9me3 [51,52]. The mechanisms leading to this silencing are not well defined, but a long non-coding antisense transcript transcribed from the provirus has been reported to silence the HIV provirus [53]. This antisense long non-coding RNA is expressed from the 3' LTR/nef region and localizes to the core promoter (5' LTR) and recruits histone modifiers such as EZH2 and HDACs to silence HIV gene expression [53]. Suppressing this lncRNA by RNAi resulted in enhanced viral gene expression and loss of repressive heterochromatin at the viral promoter. In stable ACH2 cells expressing the small antisense as154 RNA that targeted the lncRNA posttranscriptionally, there was a reduction in both H3K27me3 and H3K9me2 at the viral promoter [53]. Transducing CD4⁺ T cells with the small antisense aspro5 RNA that targeted the promoter of the lncRNA through transcriptional gene silencing also resulted in viral gene activation and reduced H3K27me3 and H3K9me2 on the 5' LTR. This suggested that the lncRNA was involved in epigenetic silencing of the HIV genome in latent infection (Figure 1). The lncRNA coimmunoprecipitated with DNA methyltransferase 3A (DNMT3a) in ACH2 and

J1.1 cells and was also found to localize to the 5' LTR. DNMT3a was shown by ChIP to occupy the 5' LTR. Mass-spec analysis showed that the lncRNA and DNMT3a were in a complex with histones and other lncRNA binding proteins. The PRC2 component, EZH2, and histone deacetylase, HDAC1, were also recruited to the 5' LTR, and reduced occupancy of these factors was observed upon inhibition of the lncRNA using as154 or aspro5 antisense RNA [53]. Further confirmation of this lncRNA and its proposed mechanisms of action by other investigators would strengthen this model.

Summary and Perspectives

It has become clear that viruses that establish a latent infection and persist in the host organism can play an active role in establishment of that infection by encoding their own gene products that modulate the viral chromatin to epigenetically silence their genome through a number of common and distinct pathways. In particular, the herpesviruses encode gene products, both proteins and RNAs, that directly or indirectly promote epigenetic silencing and latent infection. This knowledge has two implications. First, it defines a new aspect of virus-host cell interactions in which viral encoded gene products promote the down-regulation of their own expression in a cell-type or cell state-dependent manner to allow the establishment and maintenance of latent infection in specific cell types. Second, it allows the development of new strategies for latent infection therapies that target viral specific proteins. Previously, eliminating latent viral reservoirs was proposed by inducing reactivation and then inhibiting lytic infection with antivirals, such as with HIV [54]. More recently, consideration has been given to therapeutic strategies such as locking in latency. Locking in latent infection has been considered for HSV latent infection, where reactivation could occur in the nervous system and cause harm to the patient. Epigenetic drugs have been shown to block reactivation in animal models [55] and thus can be considered for a therapeutic approach for latent infection. However, epigenetic drugs may have potential toxic side effects. Therefore, therapeutics that target viral proteins that function in promoting latent infection are a very attractive new approach for the design of future drugs that prevent establishment of or reactivation from viral latent infection.

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Highlights

- Viruses such as the herpesviruses and HIV establish latent infections.
- DNA viruses undergo epigenetic silencing to establish a latent infection.
- Viruses encode their own gene products that promote epigenetic silencing of their genome.
- Viral proteins promoting latent genome silencing are potential targets for therapeutic agents.

Figure 1.
Viral gene products that play a role in epigenetic silencing during latent infection.