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Viral gene products actively promote latent infection by epigenetic silencing mechanisms

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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 the genomes and their potential for therapeutics to target these viral gene products and disrupt or lock in latent infection.

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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 *et al.* 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 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 (LAT)

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 10 kb 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 hematopoetic 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 K3K9me2, 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