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Control of Viral Latency by Episome Maintenance Proteins

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Abstract

The human DNA tumor viruses Epstein–Barr virus (EBV), Kaposi’s sarcoma-associated herpesvirus (KSHV), and human papillomavirus (HPV) share the common property of persisting as multicopy episomes in the nuclei of rapidly dividing host cells. These episomes form the molecular basis for viral latency and are etiologically linked to virus-associated cancers. Episome maintenance requires epigenetic programming to ensure the proper control of viral gene expression, DNA replication, and genome copy number. For these viruses, episome maintenance requires a dedicated virus-encoded episome maintenance protein (EMP), namely LANA (KSHV), EBNA1 (EBV), and E2 (HPV). Here, we review common features of these viral EMPs and discuss recent advances in understanding how they contribute to the epigenetic control of viral episome maintenance during latency.

Keywords

Gammaherpesvirus; KSHV; EBV; HPV; latency; episome; maintenance; epigenetics; chromatin; LANA; EBNA1; E2; oligomerization; 3D organization; segregation

Viral Episome Maintenance Proteins (EMPs): Binding and Tethering Genomes

The human DNA tumor viruses Epstein-Barr virus (EBV), Kaposi’s sarcoma-associated herpesvirus (KSHV), and human papillomavirus (HPV) persist as multicopy **episomes** (see Glossary) in latently infected cells (reviewed in [1–4]). These viral episomes are covalently closed circular genomes that are assembled into chromatin with histone and DNA modifications similar to host genomes. **Epigenetic programming** of the viral episome is important for all aspects of the viral life cycle, including the proper regulation of viral transcription during latency, cell-cycle-regulated DNA replication during latency, protection and repair of the viral genome, and the ability to switch to the lytic replication cycle in response to cell stress or differentiation signals. Each of these DNA tumor viruses encodes a dedicated episome maintenance protein (EMP) that performs a central role in viral episome maintenance and epigenetic programming. EBV EBNA1, KSHV LANA, and HPV E2 are

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structural and functional orthologs that provide essential functions, including initiation of DNA replication, regulation of viral gene expression, modulation of chromatin architecture, and tethering of viral episomes to host chromosomes during mitosis. In this review, we discuss the structural similarities of these EMPs and how this may serve as the molecular basis for epigenetic programming and episome maintenance during latent infection.

EMP DNA-Binding Domains (DBDs)

EMPs share several structural similarities, but the defining feature for this group is the structural homology between their DBDs (Figure 1). Each EMP contains a carboxy-terminal DBD and an amino-terminal chromosome-tethering domain (CTD). EBNA1 contains a repetitive glycine-alanine copolymeric stretch separating two CTD elements, while LANA has acidic repeats separating the DBD from the CTD, and E2 has a flexible hinge separating the DBD from its CTD. The DBD of each EMP directs sequence-specific binding to essential genetic elements in their respective genomes. The DBDs share a structural fold unique to these viral proteins, but not to any other known cellular protein (Figure 1A). The DBDs dimerize to form a core eight-stranded β -barrel surrounded by six α -helices with each monomer contributing four β -strands and three α -helices. DNA contacts are made primarily through amino acids in the α -helices. Additional contacts with regions extending beyond the conserved core structures also contribute to sequence-specific DNA binding. There are a number of important structural features and modes of DNA binding that distinguish each family member. One striking difference in the X-ray structures of the DNA-bound forms is that KSHV LANA binds asymmetrically to a GC-rich DNA binding site, while each monomer of the EBNA1 and E2 dimer binds symmetrically to two inverted DNA repeats [5].

EMP Function at Viral Origins of DNA Replication

A common function of EMPs is their ability to establish an **origin of DNA replication** when bound to specific sites in their respective viral genomes. EBNA1 binding to the dyad symmetry (DS) element of *oriP* constitutes a minimal origin of plasmid DNA replication [6]. A dimer-dimer interface, important for DNA replication, is formed only when EBNA1 dimers are bound with 3 bp separating each dimer at the DS [7]. EBNA1 also induces a DNA bend angle at the DS that may contribute to DNA replication activity [8]. For KSHV LANA, three tandem binding sites in the terminal repeat (TR) DNA constitute a minimal origin of DNA replication [9]. One high-affinity site nucleates the cooperative binding of the other two sites, and a positioning of 22 bp from center to center between sites 1 and 2 is necessary for DNA replication [9]. LANA also induces a strong bend in the TR DNA [10], and the bend angle is consistent with binding to the oligomeric forms of LANA that were observed in X-ray crystal studies [11]. HPV E2 also binds to multiple sites at the viral origin of replication and bends DNA [12,13]. However, unlike EBV and KSHV, E2 recruits the virus-encoded E1 helicase to initiate DNA replication during the HPV productive replication. E2 can interact with the host replication machinery, including the origin recognition complex (ORC), but it is not clear if E2 regulates origin function during the HPV latent phase [14]. Interactions with host replication proteins, including ORC, minichromosome maintenance (MCM)s, replication protein A (RPA), and proliferating cell

nuclear antigen PCNA have been demonstrated for each EMP. However, the requirement for replication initiation within these viral genetic elements bound by EMPs has been called into question. Genetic studies show that EBV episomes can be established with recombinant virus where the DS element has been deleted [15], and single-molecule analysis of replicating DNA (SMARD) demonstrated that replication can initiate at sites other than the DS in EBV [16] and the TR in KSHV [17]. Earlier genetic studies showed that the DS is required for the establishment of OriP-containing plasmids, but can be deleted after this establishment without affecting replication of the viral plasmid [18]. These studies suggest that the replication initiation function of EMPs may be critical during the early establishment phase, but may not be an essential function of EMPs in the long-term maintenance of viral episomes (Box 1).

Cooperative DNA Binding and Oligomerization of EMPs

Another shared feature between EMPs is their ability to bind cooperatively to multiple DNA sites at their viral origins of replication. The cooperative binding of LANA and EBNA1 can be partly attributed to stabilizing interactions and oligomerization potential of their DNA-binding domains. Cooperative DNA binding of LANA at TRs is critical for its stable binding *in vitro* and *in vivo* [5,19]. The LANA DBD oligomerization interface is flexible and allows for several different states, including a pentameric ring of dimers, a noncircular cluster of four dimers, or a continuous helical spiral [5,11,19] (reviewed in [1]). By contrast, the MHV68 LANA DBD forms a rigid, linear conformation, suggesting that various EMP structures can support episome maintenance [19]. The EBNA1 DBD can form a hexameric ring with an oligomeric interface that contributes to episome maintenance, but not to DNA replication function [20]. The EBNA1 hexamer interface was distinct from the dimer–dimer interface observed at the DS, suggesting that EBNA1 can exist in at least two functionally distinct oligomeric states. Cooperative DNA binding of E2 is also critical for HPV replication [21].

For each EMP, the corresponding DBD contains some unique structural features. EBNA1 has extended PG loops and N terminal arms that are critical for DNA binding and replication. The LANA DBD contains a unique patch of basic amino acids on the surface dorsal to the sequence-specific DNA-binding interface. This basic patch lines the interior of the pentameric ring observed in the LANA X-ray structures [5,11,22]. This lysine cluster contributes to nonspecific DNA binding, and mutations in this region reduced several protein–protein interactions and impaired episome maintenance [22].

Chromosome Tethering of EMPs

EMP attachment (or tethering) to metaphase chromosomes is necessary for episome maintenance and for DNA replication ([23,24] and reviewed in [1]), suggesting that these functions are mechanistically linked. Each EMP contains multiple domains with metaphase chromosome binding activity (Figure 1A). While the unifying function of this attachment is to tether the viral genomes to cellular metaphase chromosomes, the primary interaction targets for each EMP and each tethering domain appear to be different (Figure 2).

N Terminal CTD

The predominant CTD of KSHV LANA is found in the N terminus and forms an arginine-rich hairpin structure. The LANA N terminal hairpin binds to nucleosomes through an acidic pocket formed by histones H2A/H2B [25] (Figure 2). This acidic pocket is a site frequently targeted by other cellular and viral proteins, such as cellular interleukin (IL)-33 [26], human cytomegalovirus (CMV) IE1 [27], and some retroviral GAG proteins [28–30], suggesting that the H2A/H2B pocket is an attractive and frequent target for virus association with host chromosomes [31]. Cell-cycle phosphorylation of the LANA histone-binding domain alters its affinity for the H2A/H2B pocket [31], suggesting that this interaction can be subject to regulation. Surprisingly, neither EBNA1 nor E2 appear to interact with histones through this mechanism.

EBNA1 metaphase attachment occurs through two related arginine-glycine (RG) domains, each of which can bind to AT-rich DNA through direct interactions with the minor groove, similar to **AT-hook** proteins [32], or with G-quadruplex RNA [33] (reviewed in [3,34]). Furthermore, each RG domain can bind to several cellular proteins that can serve as anchors to metaphase chromosomes, including EBP2 [35], nucleolin [36], histone H1 [37], HMGA1 [38], and RCC1 [39]. Substitution of the EBNA1 N terminus with histone H1 or HMGA1 was sufficient to reconstitute episome maintenance in a short-term plasmid-based assay [37], but substitution of HMG1, which lacks an AT-hook domain and does not bind to metaphase chromosomes, was not able to maintain viral plasmids [40], highlighting the importance of AT-hook domains and **metaphase chromosome tethering** for viral episome maintenance.

E2 can also associate with metaphase chromosomes by various mechanisms [41]. BRD4, a member of the BET family of proteins, can mediate interactions with metaphase chromosomes [42]. Other protein-binding partners have also been implicated in metaphase chromosome attachment, such as the helicase ChlR1 [43] and the cohesin-like proteins SMC5/6 [44].

Other Chromosome-Binding Domains

In addition to the N terminal histone H2A/H2B interaction domain, other regions of LANA contribute to metaphase chromosome binding and episome maintenance, including the central repeats and DBD [45] (reviewed in [1]). Deletion of the central repeats reduces episome maintenance, but not viral DNA replication [45,46]. Interactions with nonhistone proteins, such as centromeric factors CENPF and Bub1, also contribute to episome maintenance [47,48]. Interestingly, the KSHV LANA N terminal histone-binding domain is not conserved in MHV68 LANA, suggesting that MHV68 LANA uses an alternative mechanism to achieve metaphase chromosome attachment and episome maintenance during latency [19].

In addition to tethering to metaphase chromosomes, EMPs can also bind to host chromosomes through sequence-specific interactions mediated by the DBDs. EBNA1 was found to interact with ~1000 specific sites on the host chromosome [49,50]. Only a few of these could be shown to have functional impact on cellular gene expression, suggesting that the majority of binding sites have unknown function. Similar observations were made for

KSHV LANA, which binds to GC-rich sequences similar to that found in KSHV TR [51–53]. LANA was found to bind and upregulate some cellular genes, including SENP6, which encodes a SUMO protease that binds and modulates LANA function during the establishment of latency [54]. While the regulation of a small cohort of cellular target genes bound by EMPs may contribute to **viral latency** and oncogenic transformation, it is also possible that the many cellular binding sites have other functions, such as facilitating viral genome attachment to host chromosomes. (Box 2).

Epigenetic Programming of Viral Episomes

Viral episomes maintained by EMPs assemble into chromatin that is epigenetically modified and structurally organized similar to the cellular chromosome. The chromatin structure and epigenetic programming are necessary for the proper control of viral gene expression and stable maintenance of viral DNA. LANA, EBNA1, and E2 have all been implicated in epigenetic programming, including regulation of replication origin function, transcriptional repression of viral lytic cycle genes, and recruitment of epigenetic modifiers [55–58].

Histone Modifications and Epigenetic Patterning

The epigenetic landscape of the EBV and KSHV genomes has been examined in significant detail [59–61]. Histone tail modifications associated with transcriptional activation (acetylated H3K9/K14 and H3K4me3) and repression (H3K9me3 and H3K27me3) can be found at various positions along the EBV and KSHV genomes during latency. Latent KSHV genomes are broadly associated with repressive H3K27me3, with the exception of the regions encompassing the major latency transcripts and the TR. A bivalent histone modification pattern with both H3K27me3 and H3K4me3 was identified at the transcriptional regulatory regions for the ORF50/Rta immediate early gene, indicating that they are poised for rapid response during viral reactivation. Similar epigenetic patterning has been detected in KS tumor biopsies [62]. In models of EBV latency, transcriptionally active promoters were enriched with H3K4me3 and H3K9Ac, but the patterns of repressive histone marks were not as broadly distributed as that found in KSHV [59]. EBV can also adopt different latency types with different extents of transcriptional repression that may be preferentially regulated by DNA methylation rather than histone modification (reviewed in [63]).

The mechanisms that determine histone modification patterns and gene promoter selection on a newly established episome are not completely understood. EMPs contribute to this programming, but many other factors, including prepackaged viral tegument proteins and cellular factors, regulate the earliest events leading to the establishment of the viral epigenome [64]. EMPs can recruit histone- and DNA-modifying enzymes that may contribute to epigenetic patterning (reviewed in [65]). LANA interacts with BET proteins BRD2 and BRD4 [22,66] that can interact with acetylated lysines, histone H3.3 chaperone DAXX [67,68], DNA methyltransferase DNMT3a [69,70], and recruits histone acetyltransferases CBP and HBO1 to KSHV TR [71]. EBNA1 can also interact with BRD4 [72], and recruit histone H3K4 methyltransferase MLL through HCF1 to OriP to facilitate replication and episome maintenance [49]. E2 interacts with BET protein BRD4 [73],

histone H3K4 demethylase SMCX, and histone acetyltransferase TIP60 [74] to regulate viral transcription. These many interactions of EMPs underscore their complex role in orchestrating various stages of epigenetic programming with episome maintenance.

EMP Autoregulation

Autoregulatory mechanisms for EMP gene expression and copy number control have been identified for each of these viruses. LANA interacts with the components of the Polycomb repressor complex (PRC2) required for generating the repressive H3K27me3 modification across the majority of the latent episome [75]. However, the LANA promoter and latency locus is spared, consistent with its continuous transcription during latency. The LANA promoter (LANAp) has remarkably simple features of a constitutively active RNA polymerase II core initiator (**INR**) element with no obvious essential transcription factors or enhancer elements[76]. ChIP-Seq data indicated that LANA can interact with its own promoter region [51–53,62]. Chromosome conformation capture (3C) analysis has identified a DNA interaction loop between the TR and the region upstream of LANAp [77]. This interaction was dependent on LANA oligomerization and was required for autoregulation of the LANA transcript and protein [77]. LANA can also interact with histone H3K9 methyltransferase SUV39H and recruit heterochromatin protein 1 (HP1) to the TR, resulting in transcriptional repression of neighboring genes [78]. Since LANA binding sites are found at TR and upstream of LANAp, it is presumed that LANA mediates these DNA loop interactions to autoregulate its own expression (Figure 3).

Related transcriptional control is observed for the EBV EBNA1 gene that can be constitutively expressed from a dedicated promoter, termed Qp. Qp utilizes a simple initiator (INR) element, and is subject to negative autoregulation by EBNA1 that binds sequence-specifically to a pair of sites overlapping this INR [79,80]. EBNA1 forms a DNA loop between DS and FR at *oriP* [81] and can also mediate interactions between *oriP* and Qp [82], suggesting a direct communication between these regulatory elements. Both LANAp and Qp are subject to autorepression [76,79], and are bound by the cellular chromatin boundary factor CTCF, which plays an important role in preventing epigenetic silencing of these critical promoters. For HPV, E2 is known to autoregulate expression of early viral transcripts for E6 and E7, as well as E2 and E1 [74]. Thus, EMPs function in autoregulation through DNA binding and DNA-looping mechanisms (Figure 3). (Box 3).

Genomic Architecture

Cellular chromatin organizing factors, such as CTCF, YY1, and cohesins, are found enriched at specific sites in these viral genomes and contribute to various aspects of viral gene regulation, including EMP autoregulation and episome maintenance. CTCF and cohesins are involved in numerous cellular chromatin functions, including boundary element, insulator, transcriptional repressor, regulator of RNA processing, and DNA loop interactions [83]. Their role in viral episome maintenance is multifunctional and complex, as it is for the cellular epigenome. For HPV, CTCF and YY1 regulate viral gene expression through a DNA loop [84], and cohesin subunit SMC1 has been implicated in chromosome tethering and DNA damage repair during replication [85]. For EBV and KSHV, CTCF and cohesins have been implicated in transcription control, suppression of epigenetic drift and 3D architecture,

including loop formation between DNA regulatory elements (reviewed in [63]). Disruption of loops mediated by CTCF and poly (ADP-ribose) polymerase 1 (PARP1) in EBV leads to a switch between latency type I and III [86], while in KSHV depletion of cohesin subunits RAD21, SMC1, or SMC3 activates lytic cycle gene expression in latently infected plural effusion lymphoma (PEL) cells [87,88]. Thus, viral DNA loops and chromosome architecture are important for maintaining stable gene expression programs in latency.

Genome-wide DNA interaction assays, such as HiC, revealed an extensive web of interactions for KSHV, with strong clustering of lytic cycle gene promoters with the PAN gene locus during lytic cycle reactivation [89]. The findings suggest that RNA polymerase remains poised at the PAN promoter during latency and is involved in extensive remodeling of the KSHV genome during reactivation. KSHV genomes colocalize with RNA polymerase-associated factories during lytic cycle reactivation [90]. HiC analysis of the EBV genome revealed an association of the viral genome with heterochromatic domains during latency, and a shift towards euchromatic regions during reactivation [91].

Higher-Ordered Episomal Structures in Nuclear Bodies

EMPs and viral episomes can form various structures in the host cell nucleus as visualized by fluorescent-light microscopy. For each virus, the formation, localization, and mobilization of these structures appear different. These structures are almost always associated with metaphase chromosomes but may have additional colocalizations during interphase. Here, we consider aspects of EMP function in forming higher-ordered structures and how this contributes to episome maintenance and protection.

Episome Nuclear Bodies Resist Promyelocytic Leukemia (PML) Nuclear Bodies

There is complex interplay between nuclear viral genomes and the host nuclear intrinsic antiviral resistance structures formed by **PML-nuclear bodies (PML-NBs)**. Latent episomes of EBV are associated with metaphase and interphase chromatin, and do not colocalize with PML-NBs [92]. However, EBNA1 can interact with PML and disrupt its function during the lytic phase [93]. LANA, in association with viral episomes, forms large discrete structures, termed LANA nuclear bodies, that colocalize with metaphase and interphase chromosomes [94]. DAXX, a typical component of PML-NBs, interacts with LANA and colocalizes with LANA bodies by IF imaging [67,68]. Interestingly, LANA bodies do not colocalize with PML-NBs, indicating that they are distinct structures from PML-NBs [77,95]. LANA has been shown to redistribute SP100, another PML-NB component, into different subcellular compartments [67]. LANA bodies partly colocalize with ORC and are enriched for H3K27me3 and PRC components [75,77]. HPV E2 was also shown to colocalize with PML-NBs [96]. Subsequent studies have implicated PML in HPV transcription activation [97], and SP100 in restricting viral transcription and replication [98].

Episome Bodies during Mitosis

Several studies have addressed the localization of EMPs and viral episomes to specific regions of the metaphase chromosome. For HPV, most E2 proteins, with the exception of the alpha family members, remain associated with ribosomal DNA and pericentromeric regions

of metaphase chromosomes throughout mitosis [41,99]. For KSHV, LANA foci on metaphase chromosomes were found to be mostly random, with some enrichments at pericentromeres, peritelomeres, and furrows between cohering sister chromatids [100]. LANA interacts with centromere regulatory protein Bub1 to block phosphorylation of H2A [47], and can regulate centromere protein biology, suggesting that tethering at these sites may involve pirating centromeric functions. For EBV, EBNA1 foci on metaphase chromosomes appear to be randomly distributed but symmetrically dividing during chromatid separation [101]. At least one study found an interaction between EBNA1 and the kinetochore component Survivin, suggesting that a coordination with centromere regulatory factors may also occur with EBV EBNA1 [102].

Live-cell imaging studies of KSHV LANA have been controversial. In one study, LANA bodies formed stable structures throughout mitosis. They underwent considerable movement and reorientation during chromosome congregation, and segregated with near equal distribution to each daughter cell to form a pattern nearly identical to that of the parent cells [77]. A different imaging system utilizing the LACi repressor system found that KSHV genomes did not segregate faithfully, but rather formed large clusters that segregated asymmetrically with large copy number increases in one of the two daughter cells [103]. These discrepancies may be due to different experimental models, but may also reflect multiple mechanisms of episome segregation for the same virus and EMP.

Super-resolution microscopy of LANA bound to 2xTR plasmids revealed important structural information on how LANA interacts with the TR [104]. This study revealed that the TR chromatin conforms to a euchromatic active state with the ordered tethering domain and the internal repeats forming a coiled-coil. The LANA N termini appear to be oriented in a way that facilitates exploration of the surrounding environment to promote further tethering.

Episome Maintenance and Phase Separation

There has been recent interest in self-organizing structures, such as viral replication compartments, and phase-separation biochemistry [105]. Whether stable episomes, such as those associated with LANA-NBs, result from phase separation is not yet known. RNA polymerase transcription factories and super-enhancers have been associated with phase transitions [106,107]. The formation of RNA pol II factories during KSHV reactivation [90], EBNA2-induced super-enhancers [108], and LANA-NBs may also be different forms of phase transition regulating structure and function of viral episomes [77].

Concluding Remarks

Episome maintenance is an essential part of the life cycle of the persistent DNA tumor viruses KSHV, EBV, and HPV. Disruption of episome maintenance can deregulate many aspects of the viral life cycle, including gene expression, DNA replication, and genome segregation. Several efforts to inhibit EMPs have been explored, including inhibitor assays of the LANA histone interaction domain [109], small-molecule inhibitors of the EBNA1 DNA-binding domain [110–112], and peptide inhibitors of E2 functions [113,114]. CRISPR/CAS9 genome editing of KSHV LANA [115], or EBV OriP [116,117] can reduce

or eliminate viral episomes in cell culture models. Further understanding of the structure, function, and biology of these EMPs will provide important new insights for improving these potential therapeutic strategies (see Outstanding Questions).

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Glossary

AT-hook

a protein domain, such as that found in HMGA1 and EBNA1 tethering domains, that bind to the minor groove of AT-rich DNA.

Epigenetic programming

epigenetics describes the information that can be transmitted between generations above the primary DNA sequence. Latent episomal viruses contain epigenetic information for regulating transcription, DNA replication, segregation, and genome integrity. EMPs are thought to help establish epigenetic programming during viral latency.

Episome

although originally named for mobile DNA elements that can both integrate and form circular DNA molecules, the term is commonly used in the Gammaherpesvirus field to describe the covalently closed circular form of EBV and KSHV genomes that remains unintegrated and competent for viral transcription and replication during latency.

INR

initiator element involved in directing RNA polymerase to start transcription. The INR is one of several genetic elements that determines transcriptional start sites. They are found at the start sites of the KSHV LANA and EBV EBNA1 genes.

Metaphase chromosome tethering

episomal viral genomes attach to metaphase chromosomes to persist in dividing cells. Failure to attach results in loss of episomes. The EMPs mediate interactions between the viral genome and host metaphase chromosome. This tethering mechanism appears to be varied and different for each virus.

Origin of DNA replication

herpesviruses, such as EBV and KSHV, have genetic elements that confer the capacity to initiate DNA replication during latency. The *oriP* for EBV and the terminal repeats (TRs) of KSHV can function as origins of DNA replication during latency. In addition to these latency-associated origins, the viruses have a separate and dedicated origin of lytic replication (*oriLyf*) that requires virus-encoded DNA replication enzymes and produces many viral genomes through a rolling-circle mechanism. Papillomaviruses also have latent and lytic replication cycles but utilize the genetic element for these different replication mechanisms, both mediated by E2.

PML-NB

promyelocytic leukemia (PML)-nuclear bodies (NBs) are punctate nuclear structures that form in response to many viral infections. They restrict viral transcription and DNA replication through chromatin-based mechanisms. Many viruses encode factors that destroy PML-NBs or hijack their functions for viral purposes, such as establishment of latency.

Viral latency

the state of infection where no infectious virus is produced. For the DNA viruses discussed here, limited viral transcription occurs during latency, and can be required for maintenance of the latent state in proliferating cells. Viral DNA can replicate using host replication machinery, but is not packaged into infectious virions.

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Box 1.**Episomes in Natural Infection and Cancer**

Episomal forms of KSHV, EBV, and HPV are found in most of the corresponding virus-associated cancers (reviewed for KSHV [118–120], EBV [121], and HPV [122]). In normal carriers, EBV episomes persist in long-lived memory B cells [123,124], and HPV episomes persist in basal epithelial cells [125]. While integrated viral DNA can be found in the host genomes of cell lines and tumors, these are almost always aberrant genomes that fail to produce infectious virus upon reactivation [126,127]. Aberrant integration can lead to deregulated expression of viral and cellular oncogenes, and this is a common oncogenic mechanism for Merkel cell carcinoma and a subset of HPV-associated tumors [128,129]. Long-term genome maintenance may also require sporadic productive infection [130]. In most model systems, the efficiency for the establishment of a stable viral episome is very low and requires positive selection [130–132]. Therefore, these viruses must provide a selective advantage to the cells and tumors that maintain stable episomes. Evidence suggests that epigenetic events in the cell and on the viral genome must occur to establish and maintain a stable episome [133,134].

Epigenetic and transcriptomic analyses of tumor tissue reveal important differences compared with cell culture models. The epigenetic landscape of KSHV in KS tumor tissue was found mostly similar to KSHV in latently infected PEL and SLK cell lines [62]. The overall enrichment of H3K27me3 was similar, but histone acetylation was restricted to the latency locus, including the LANA promoter and the K12 transcripts, with no significant acetylation in the TR or vIRF3 locus in tumor-associated KSHV genome [62]. RNA-seq studies from KS tissue revealed some differences from cell culture models [135,136]. In one study, viral gene expression was found to be a mix of both latent and lytic transcripts, suggesting a heterogeneous population with some small percentage of cells in the lytic cycle [136]. In a second study, with more samples and focusing primarily on viral transcripts, viral transcript patterns in the latency-control region were significantly different than those reported in latently infected PEL and SLK cells, with unusually low levels of transcripts initiating at the LANA promoter and high levels of transcripts initiating from a downstream promoter driving viral miRNAs and Kaposin A [135]. Another recent study of naturally occurring infections revealed that KSHV genomes have different subtypes, and these undergo extensive recombination [137].

Box 2.**Dynamic Changes in EMPs**

Variant forms of EMPs have also been identified. A splice variant containing the E2 DBD, termed E8^ΔE2, can function as a negative regulator of HPV transcription and replication [138]. Smaller forms of LANA have been shown to function in the cytoplasm [139,140]. Smaller isoforms of LANA have been identified in the cytoplasm, and interact with and downregulate the stimulator of interferon genes (STING)-cyclic GMP-AMP synthase (cGAS) pathway of innate immune signaling [2,140]. Another potential function of the cytoplasmic forms of LANA could be to protect the viral genomic material during nuclear membrane breakdown in mitosis.

Box 3.**Potential Interactions between EMPs**

Coinfections of DNA tumor viruses can occur in some tumor types, and the potential interactions between viral proteins is important to consider. EBV and KSHV are frequently found as coinfections in pleural effusion lymphoma (PEL). In dually infected PEL cells, KSHV episome maintenance was found to be codependent on EBNA1 and EBV episome maintenance [141]. Although each viral episome was found as distinct and separate foci by IF, a CRISPR deletion of EBNA1 led to a corresponding loss of both EBV and KSHV episomes. EBNA1 was sufficient to enhance KSHV episome maintenance, potentially through an indirect effect on the host cell environment. Thus, EMPs may influence the functionality and cell permissivity for other EMP family members.

Highlights

Viruses have evolved elaborate mechanisms to maintain their genomes as passengers in host cells.

The oncogenic human herpesviruses, EBV and KSHV, and the small DNA tumor virus, HPV, persist as multicopy episomes.

EBV, KSHV, and HPV have dedicated EMPs that bind their viral genomes and tether to cellular metaphase chromosomes.

EMPs contribute to the epigenetic programming of latent viral genomes.

Outstanding Questions

- How do oligomeric forms of EMPs relate to higher-ordered viral chromosome structure and functional organization? Are the different geometric forms, such as hexamers and pentamers, related to different functions in the viral life cycle?
- How do episome maintenance proteins function to establish origins of DNA replication and centromeric segregation functions?
- Do EMPs form a physical shell that protects the viral genomes and facilitates their mobilization through nuclear compartments and during chromosome segregation?
- Are viral episome bodies formed through biophysical forces of liquid-phase change or some other forces of biomolecular self-assembly?
- Will viral EMPs serve as good targets for antiviral therapies?

Figure 1.

Episome Maintenance Proteins (EMPs). (A) Schematic structure showing C terminal DNA-binding domain (DBD) in red, and the various N terminal domains involved in chromosome tethering, DNA replication, and transcription-activation domains (TADs). (B) Co-crystal structures of EMPs KSHV LANA (red) (PDB 4UZBP), EBV EBNA1 (yellow) (PDB 1B3T), or HPV E2 (cyan) (PDB 1JJ4) bound to cognate DNA elements. (C) Oligomeric structures of EMPs including LANA decameric ring (PDB 4KJ2) and EBNA1 hexameric ring (PDB 5WMF). Abbreviations: KSHV, Kaposi's sarcoma-associated herpesvirus; EBV, Epstein-Barr virus; HPV, human papillomavirus.

Figure 2.

Metaphase Chromosome Attachment Mechanism. (A) Crystal structures of episome maintenance protein (EMP) interactions with targets in metaphase chromosome attachments. LANA (red) N terminal peptide bound to histone H2A-H2B pocket of the nucleosome (PDB 1ZLA), HPV E2 peptide bound to BRD4 (cyan) (PDB 2NNU), and an AT-rich minor-groove binding molecule netropsin with DNA representative of the EBNA1-AT hook interactions with metaphase chromosomes (PDB 1Z8V). (B) Partial list of metaphase tethering targets for EMPs. LANA interacts with BRD4, DEK, H1, Bub1, MeCP2; E2 interacts with BRD4 and ChIR1; and EBNA1 interacts with AT-rich DNA, RNA-G quadruplex, and protein-target (EBP2, nucleolin, HMGA1, H1). (C) Depiction of the viral episome (small black rings) bound to metaphase chromosome positions by LANA (red) at peritelomeric and pericentromeric regions, E2 (yellow) bound to pericentromeric and rDNA regions, and EBNA1 (blue) bound to AT-rich DNA elements. Abbreviations: KSHV, Kaposi's sarcoma-associated herpesvirus; EBV, Epstein-Barr virus; HPV, human papillomavirus.

Figure 3.

Architectures of Viral Epigenomes. Episome maintenance proteins (EMPs) mediating autoregulatory interactions to control epigenome architecture and regulation of gene expression during latency. Kaposi's sarcoma-associated herpesvirus (KSHV) (top left), Epstein–Barr virus (EBV) (top right), human papillomavirus (HPV) (lower left), and protein key (lower right). Abbreviations: DS, dyad symmetry; ORC, origin recognition complex; PRC, Polycomb repressor complex; Qp, promoter.