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Epigenetic Regulation of EBV Persistence and Oncogenesis

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Abstract

Epigenetic mechanisms play a fundamental role in generating diverse and heritable patterns of viral and cellular gene expression. Epstein-Barr Virus (EBV) can adopt a variety of gene expression programs that are necessary for long-term viral persistence and latency in multiple host-cell types and conditions. The latent viral genomes assemble into chromatin structures with different histone and DNA modifications patterns that control viral gene expression. Variations in nucleosome organization and chromatin conformations can also influence gene expression by coordinating physical interactions between different regulatory elements. The viral-encoded and host-cell factors that control these epigenetic features are beginning to be understood at the genome-wide level. These epigenetic regulators can also influence viral pathogenesis by expanding tissue tropism, evading immune detection, and driving host-cell carcinogenesis. Here, we review some of the recent findings and perspectives on how the EBV epigenome plays a central role in viral latency and viral-associated carcinogenesis.

Keywords

Epstein-Barr virus; gammaherpesvirus; chromatin; histone modifications; DNA methylation; chromosome conformation; CTCF; OriP

Introduction

Epstein-Barr virus (EBV) is a human gammaherpesvirus that can establish a life-long infection in 95% of the population worldwide [1]. The remarkable success of this viral pathogen can be partially attributed to its ability to establish a variety of gene expression programs that enable adaptation to different cell types and host-cell conditions. Variation in viral gene expression may also account for the broad range of viral-associated disease. EBV

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genomes and gene products are consistently detected in a diverse number of human cancers, including endemic Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), ~50% of Hodgkin's disease, ~10% of gastric carcinomas, and most lymphoproliferative disorders of immunosuppressed individuals [2, 3]. In each of these cancer-associated infections, EBV has a distinct gene expression program that reflects the host cell-type transcription factors, and ultimately, distinct epigenetic modifications of the viral genome. Epigenetic modifications are thought to generate diversity, as well as provide stability to gene expression programs in dividing cell populations. Here, we review how epigenetic modifications and chromatin organization play a central role in generating both diversity and stability of EBV gene expression programs during latent infection in various normal and cancer cell types.

EBV Latency Types

During latent infection, most EBV genomes persist as circular minichromosomes in the nucleus of infected cells (Fig. 1A) [1]. EBV gene expression during latency is highly restricted compared to the productive lytic cycle, and depends on the tissue or tumor type from which the EBV-positive cell-line was derived [4]. At least four different gene expression programs have been described, and are referred to as latency types [5]. In lymphoblastoid cell lines (LCLs) and B-cell lymphomas that occur during immunosuppression, EBV expresses the full set of latency associated genes. This least restrictive latency type is referred to as Type III latency, and consists of the expression of EBNA1, -2, -3A, -3B, -3C, -LP, the latency membrane proteins LMP1 and -2, and the non-coding RNAs (the EBERS, microRNAs, and the BARTs) [6]. All other latency types involve increasing degrees of viral gene silencing. For example, Type I latency consists of the expression of only one viral protein, EBNA1 and a few non-coding RNAs [7, 8]. The different latency types and their corresponding gene expression programs correlate with alternative utilization of transcription start sites and promoter elements [5, 9] (Fig. 1B). For example, in Type I latency, the EBNA1 gene is transcribed from the EBV Q promoter (Qp) localized in the BamHI Q region of the EBV genome [10], while in type III latency, a polycistronic mRNA coding for all the EBNA genes is initiated from the C promoter (Cp) in the BamHI W/C region of the EBV genome [5]. Also, promoter switching occurs during EBV immortalization and B-cell maturation, with transcription initiating at the W promoter (Wp) during primary infection and its subsequent switching to the upstream start sites controlled by Cp. The mechanisms that control promoter selection and switching during B-cell maturation are not completely understood. EBNA2 protein is required for strong activation of Cp, as well as LMP1, and its expression and function is closely coordinated with B-cell identity and proliferation factors [9, 11]. Some of the central players in B-cell development, like Pax5, Pu.1, and RBP-jK, are known to play similarly central roles in regulating EBV promoter function and latency type. Dynamic feed-forward and auto-repression mechanisms contribute to the establishment of a stable gene expression program for each latency type [12]. These programs are further reinforced by epigenetic changes on the viral genome.

Epigenetic Control of EBV Latency

Epigenetic programming plays a central role in regulating EBV gene expression and latency types [13]. Epigenetic programming involves DNA methylation, nucleosome positioning, histone tail modifications, and higher-ordered structures including promoter-enhancer loop formation. The importance of epigenetic modifications in the control of EBV latency has been known for over a decade as it has been well-established that pharmacological inhibitors of DNA methylation and histone deacetylation can both activate Type III promoters from Type I cells, as well as reactivate lytic gene transcription in some latently infected cell types [14, 15]. In latently infected cells, the majority of EBV genomes exist as non-integrated episomes with a nucleosomal pattern similar to that of host chromatin [16, 17]. Nucleosome position and density has been implicated in the regulation of most host DNA processes, and is also likely to regulate the transcription, replication, and repair of the latent EBV genomes. DNA methylation is typically associated with transcriptional repression, although there are many exceptions and EBV lytic activation is one of these exceptions [18]. Post-translation modifications of histone tails are among the best characterized epigenetic modifications, and can alter the chromatin structure in a reversible way that controls access to DNA [19]. Modifications of histones have complex and sometimes paradoxical roles in regulation of gene activity. In general, acetylation of Lysine 9 (H3K9ac) and methylation of Lysine 4 of histone H3 (H3K4me) mark regions of chromatin associated with active gene expression [19, 20]. In addition, the extent of lysine methylation can distinguish between different regulatory features: H3K4me1 marks enhancers; H3K4me2 marks both enhancers and promoters; H3K4me3 marks promoters and transcription starting sites (TSS) [21, 22]. Histone modifications associated with heterochromatin include methylation of lysine 9 (H3K9me3) [23, 24] and lysine 27 of histone H3 (H3K27me3) [25]: H3K9me3 marks regions associated with constitutive heterochromatin and repetitive elements; H3K27me3 marks regions repressed by polycomb complex, indicative of developmentally regulated genes [25]. Recent genome-wide studies have revealed that the EBV is subject to complex patterning of these histone modifications and that the patterns vary between latency types and correlate with different viral gene expression programs [26].

Histone Modifications on the EBV Genome

The chromatin composition of the EBV genome varies between different latency types and the histone modifications tend to correlate with expected marks at sites of transcriptional activation or repression. A high level of epigenetic variability between Type I and III latency occurs at the transcription start sites for the Type III-specific viral genes. The Cp and the LMP1/LMP2 promoters are enriched for acetylated histones (e.g. H3K9Ac, H3K27ac, H4Ac) as well as for H3K4me3 in type III latency where they are actively transcribed. These euchromatic marks are absent from Cp and LMP1/LMP2 in Type I latency where these promoters are inactive [27]. In contrast, the Qp is associated with transcription activation marks, such as H3K9Ac, H4Ac, H3K3me2 and H3K4me3 in Type I latency, where Qp is active, but not in Type III, where Qp is repressed [28, 29] (Fig. 2). Histone modifications associated with constitutive and facultative heterochromatin are also varied in different latency types. In Type I latency relatively high levels of H3K9me3 are observed at the W repeats, the Cp and the LMP1/2 promoters, which are transcriptionally repressed. In

contrast, Type III infected cells show a general low level of H3K9me3 across the viral genome, as might be expected since all the latent genes are expressed [29, 30]. Interestingly H3K27me3 is weakly enriched at the lytic immediate early promoters in both Type I and Type III latency, suggesting that facultative heterochromatin associated with polycomb complex repression limits lytic reactivation [29, 31, 32]. H3K27me3 and polycomb-associated repression has been implicated in the control of KSHV latency, and may serve a similar function in EBV latency, as well [33, 34].

DNA Methylation of the EBV Genome

DNA methylation is known to play an important role in controlling cellular and viral gene expression. DNA methylation of promoter regions correlates with gene silencing and regulates several cellular processes including genomic imprinting, suppression of transposable elements, and X-chromosome inactivation [35-37]. In EBV DNA methylation levels has been explored both in Type I and III latently infected cell lines [38-40]. Following the infection of primary B-cells in vitro the EBV genome becomes progressively methylated at different regions including the W repeats, the C and the LMP1/2 promoters and sites for the initiation of transcription for lytic genes [28, 39, 41-44]. Naïve B-cells from healthy EBV positive donors as well as primary tissues from different EBV-positive lymphomas show similar DNA methylation pattern across the viral genome [45]. Throughout the EBV genome DNA methylation frequency overlaps with heterochromatic marks for H3K9me3, and correlates inversely with euchromatic histone modifications H3K9ac and H3K4me3. This is consistent with the reported interactions of DNMTs with histone H3K9me3, and underscores the cross-talk between epigenetic modification [46].

Several studies show that CpG methylation levels are elevated at the Cp and LMP1 promoters in Type I latency relative to Type III [28, 47, 48]. Treatment with the DNMT inhibitor 5-azacytidine can revert the restricted gene expression program in Type I latently infected cells, resulting in the reversal of methylation at the Cp and LMPs promoters, and the transcription reactivation of EBNA5, LMP1 and LMP2 mRNAs [15]. This clearly demonstrates the need for active DNA methylation to maintain stable repression of Cp and LMPs promoters during Type I latency. While DNA methylation can silence Cp and LMP promoters, DNA methylation is never observed at the Qp, despite the fact the Qp may be silenced in Type III latency [28, 43]. However, the Qp can be methylated when removed from its EBV genome context and inserted into a reporter expression constructs [49] indicating that Qp is protected from DNA methylation in the context of the viral genome.

How different methylation patterns are established between latency types it is not known. The viral protein LMP1 downregulates the expression of DNMT1 in germinal center B cells infected with EBV [50]; and DNMT1 and DNMT3B are upregulated in EBV positive cell lines supporting Type I latency compare to Type III latency [51]. However, the depletions of both DNMT1 and DNMT3B failed to reactivate lytic gene expression from the Type III latency or to induce Cp reactivation from Type I latency [51]. However, other studies have found that 5' azacytidine can reactivate Cp transcription from some Type I cells [52]. These different findings may reflect different cell types and viral epigenotypes that respond differently to perturbations in epigenetic modifications.

DNA methylation also plays a central role in regulating EBV lytic cycle gene expression. While DNA methylation is typically associated with transcription repression, methylation of some viral promoter elements is essential for lytic cycle reactivation. This paradoxical effect is explained by the ability of the viral-encoded immediate early protein Zta (also referred to as ZEBRA, Z, and BZLF1) to selectively bind to methylated DNA at a subset of viral lytic promoters [53, 54]. EBV genomes are gradually methylated during the establishment of latency, but it is the lack of DNA methylation that prevents the completion of the viral lytic cycle. DNA methylation is required for Zta to bind promoter DNA of several essential viral genes [55, 56]. Zta has evolved the specialized capacity to overcome the silencing of lytic genes by selectively binding to methylated DNA [56, 57]. Interestingly, the Zta promoter is silenced by epigenetic mechanisms distinct from DNA methylation, including sequence specific repressor proteins like ZEB1 [58], and elevated H3K9me3 repressive marks that prevent lytic gene expression and replication during latency [30, 31]. Thus, DNA methylation plays both negative and positive roles in regulating EBV gene expression programs.

Chromatin insulators and three-dimensional structure of the EBV episome

Recent genome-wide ChIP-Seq studies of latently infected cells reveals that EBV genomes have complex and mottled epigenetic patterns [26]. In some cases, epigenetic modifications form a domain with a distinct boundary marked by the CCCTC-binding factor, CTCF [28]. CTCF is a highly conserved 11-zinc finger DNA binding protein that regulates different aspects of chromatin organization [59]. CTCF plays a crucial role in cellular processes such as gene expression, enhancer blocking, gene imprinting, and chromatin insulation [60, 61]. CTCF binding profile on EBV genome identified 19 CTCF binding sites, most of them at key regulatory regions including the Cp, the Qp and the LMP1/2 promoters [26, 28, 62]. The precise function of CTCF binding at a specific loci is not always obvious since CTCF can have multiple functions and operate at distal locations [60]. However some of the CTCF sites are situated perfectly to function as a border between epigenetic domains, suggesting that at these loci CTCF could function as a chromatin boundary factor [26, 28]. This is the case of the Qp promoter where CTCF binds upstream the transcription start site, bordering on a chromatin region enriched for H3K9me3 and DNA methylation (Fig. 2). When CTCF binding is removed by genetic manipulation of EBV genome the repressive chromatin marks can spread into the Qp start site and silence transcription [28]. The role of CTCF upstream of the Cp is less clear. Since CTCF binds between the Cp and OriP, which is known to work as a distal enhancer for this promoter, it has been proposed that CTCF could function as insulator and then negatively regulate Cp activity in Type I latently infected cells [63]. This model seems to be supported by the higher occupancy of CTCF at Cp in Type I compared to Type III latency [28, 63]. However global depletion of CTCF failed to reactivate Type III expression program in Type I latency cells, and CTCF binding was not universally reduced at Cp in all cell types tested [51, 64]. These findings suggest that CTCF binding at Cp may not be a simple blocker of OriP-enhancer function, but may play a more complex role in regulating latency type gene programming, including the formation of different higher-ordered chromatin structures [51].

CTCF contributes to the three-dimensional organization of genome by promoting long-distance interactions between different DNA regions [60, 65, 66]. Genome-wide analysis of chromatin architecture in human cells revealed that long-distance interactions usually involve enhancers and promoters and correlate with gene expression activation [67, 68]. Three-dimensional analysis of EBV genome revealed that chromatin loops are established between the enhancer region OriP and either the Qp in Type I or the Cp in Type III [69]. The integrity of the CTCF binding sites upstream of Cp and Qp is critical for the formation and the maintenance of these chromatin loops [69] (Fig. 3). However the disruption of the loops did not result in the immediate loss of promoter selection and gene expression [28, 51, 63, 69]. Rather, the loss of Qp expression required additional time for its eventual silencing by DNA methylation. These observations suggest that EBV promoter selection and latency switching are hierarchically organized processes regulated by chromatin composition, chromatin folding, and transcription factor binding at key regulatory regions, including OriP. At present we do not know how CTCF binding sites form selective chromatin loops in one type of latency but not in another.

The association between CTCF and other cellular or viral factors may also contribute to alternative long-range interactions by stabilizing specific chromatin loops. CTCF can interact with members of the cohesin subunits SMC1, SMC3 and RAD21 [70-72]. The cohesins have essential roles in sister-chromatid cohesion and transcription regulation, and function by forming a ring structure that can link different molecules of DNA [73-75]. Interestingly, cohesins colocalize with some, but not all of the CTCF binding sites, suggesting that at least some CTCF sites have specialized DNA looping functions [26, 62]. Recently, the knockdown of cohesin subunits in Type III latently infected cells resulted in a reduction of LMP1 and LMP2 expression and in the disruption of the long-range interaction between OriP and the LMP1/LMP2 promoter [26]. These findings suggest that a complex network of interactions regulates EBV gene expression. Understanding how these long-range interactions are regulated will expand our knowledge of the epigenetic factors that control EBV gene expression during infection and carcinogenesis.

OriP as a chromosome organizer of EBV

One common epigenetic feature of Types I and III latency is the enrichment of euchromatic marks surrounding the origin of plasmid replication (OriP) and RNA pol III EBER transcripts [17, 26, 29, 76]. The chromatin structure of OriP is thought to be critical for the correct function of OriP as origin of replication, but it is also likely to be important for its function as a transcriptional enhancer of Cp and LMP1/LMP2 in Type III latency. EBNA1 binds to OriP in all latency types and EBNA1 can affect nucleosome phasing and DNA conformation at the FR region of OriP [77]. OriP also interacts with the chromatin remodeling protein SNF2h [76] and components of the origin recognition complex (ORC) that associate with chromatin modifying proteins including the histone acetylase HBO1 and the heterochromatin protein 1 (HP1) [78, 79]. How these factors influence OriP chromatin structure and histone modification patterns, and how these may regulate latency type transcription remains an important area of investigation.

Recent studies have implicated OriP as a central hub in long-range DNA interaction [26, 69]. Long-range interactions between DNA regulatory elements occur frequently in higher eukaryotes and play important roles in gene regulation. Recently genome-wide analysis of the spatial organization in different human cell lines identified more than 1000 long-range interactions between TSS and regulatory elements, including enhancers, promoters and CTCF-binding sites [67]. The spatial analysis of EBV genome showed that OriP can establish long-range interactions with several latency promoters including Cp and LMP1/2 promoters in Type III and Qp in Type I, supporting the idea that OriP is essential for viral promoter activation during latency. The family of repeats (FR) region of OriP has been shown to function as an enhancer of the Cp transcription [80-84]. The three-dimensional organization of EBV episomes suggest that OriP can also function as an enhancer of LMPs in Type III, and Qp in Type I latency [26, 69]. 3C studies indicate that OriP can physically interact with these promoter elements with the intervening DNA looping out, indicating that different episome conformations exist for different latency types. In these studies, OriP appears to have a central organizing role for EBV chromatin structure (Fig. 4).

The region encompassing the LMP1/2 promoter, EBERs transcription units, and Cp promoter has been proposed to function as a locus control region (LCR) in analogy with cellular β -globin LCR [52, 85, 86]. LCRs are characterized by a strong-enhancer activity, initiation sites for DNA replication, chromatin domain-opening activity, and enrichment for open chromatin marks such as H3Ac and H4Ac [87]. The OriP region of EBV shares most of the LCR properties including enhancer activity and initiation of DNA replication [85]. Cellular LCRs can engage in chromatin looping with the linked genes to form an active chromatin hub (ACH) [88]. OriP can serve as an ACH by mediating multiple interactions with viral and possibly cellular promoter regions. The DS and the FR elements of OriP can also form a DNA loop mediated by EBNA1, suggesting that the spatial organization of OriP involves multiple and overlapping DNA interactions [89]. As for the β -globin ACH [66], CTCF binding sites have been implicated in mediating multiple and overlapping long-range interactions [28, 62]. Based on these observation we suggest that the region encompassing OriP folds to form an ACH and LCR that coordinates the latency type promoter selection and transcription programming.

EBV as an epigenetic regulator of the host chromosome

EBV latent episomes and latency gene products can also influence the epigenetic state of the host genome (summarized in Table 1). For example, EBNA2 interacts with host cell sequence-specific binding factors, like RBP-jK and Pu.1, and transcriptional coactivators, like p300, CBP and PCAF, to modulate both viral and host gene expression [90]. These transcriptional co-activators are histone acetyltransferases (HAT) [91-93] and allow EBNA2 to direct epigenetic reprogramming of the viral and host chromosome. EBNA2 interacts also with hSNF5/In, a member of the Swi/Snf family, which is involved in the nucleosome remodeling [94]. EBNA2 can also recruit the RNA polymerase II elongation factor pTEFb to facilitate the elongation of the large polycistronic EBNA transcript initiating from Cp, an event that can alter local and global chromatin structure of target genes [95]. Presumably, EBNA2 modulates host chromosome sites in a similar fashion.

EBNA3C has been shown to repress host tumor suppressor genes through the recruitment of polycomb complex and the formation of histone H3K27me3 [96]. The polycomb repressive complex (PRC) participates in the formation of heterochromatin on developmentally regulated genes, and functions as an important modulator of tumor suppressor gene activity in human carcinogenesis [97]. Ezh2 is a member of the PRC2 complex and can also function in the repression of the EBV genome during latency. Ezh2 binds to the Zta promoter in some latency types to catalyze H3K27me3 associated transcription silencing [31]. It is not yet known whether EBNA3C is responsible for the epigenetic silencing of the EBV genome, in addition to its repression of select genes in the host chromosome.

EBV latent infection has also been linked to the increase DNA methylation of host tumor suppressor genes, especially in nasopharyngeal carcinoma (NPC) [98] and gastric carcinomas (GC) [99, 100]. The viral gene products and mechanisms responsible for site selective DNA methylation of host tumor suppressor genes are not completely clear. LMP1 can activate DNMT1 through the JNK-AP-1 signal transduction pathway in NPC [101], while LMP2A can induce DNMT1 activation in gastric carcinoma through STAT3 signaling [102]. EBV encoded miRNAs are highly elevated in epithelial carcinomas and may target cellular genes important for host epigenetic programming, including DNA methylation patterning [103]. EBNA1 has been implicated in altering DNA methylation patterns at the EBV genomes [104], and can bind to the host cell chromosome at specific sites, but has not yet been shown to affect host cell DNA methylation. Identifying the viral factors and the mechanisms controlling site specific DNA methylation will be necessary for understanding the mechanism of EBV carcinogenesis in epithelial cell carcinomas.

EBV latent infection may remodel the host chromosome structures. Recent studies have shown that immortalization of primary B-lymphocytes alters host chromosome telomeres [105]. Telomere associated DNA damage signals are linked to changes in telomere end-protection and chromatin structural alterations. Ectopic expression of EBNA1 alone was sufficient to produce telomere dysfunction in transformed B-cells, indicating the EBNA1 contributes to telomere dysfunction during EBV latent infection [106]. EBNA1 can bind to numerous sites on the host chromosome [107-110] and may alter chromatin structure or nucleosome positioning at those sites [111]. On the EBV genome, EBNA1 can alter the nucleosome positioning and mediate DNA loop formation [112, 113], so it would not be unexpected to induce similar effects at EBNA1 binding sites on the host-chromosome. Recently it has been demonstrated that EBNA1 binding to the host genome promotes a global change in chromatin organization that results in more open chromatin structure [114]. EBNA1 binding to host chromosomes can alter host cell gene expression [115], but may also function to select chromosome attachment sites for viral episome tethering during mitosis. Thus, EBNA1 may function to alter host chromatin structure to favor viral genome tethering to euchromatic regions of the host chromosome.

Outlook

Epigenetic mechanisms provide a complex and robust level of control of gene expression and genome organization. For mobile genetic elements, like DNA tumor viruses, epigenetic mechanisms play a central role in the rapid assembly and disassembly of viral genomes into

functional chromosome-like structures. This is especially significant for EBV, which adopts a variety of gene expression programs and persist in diverse cell types. Epigenetic modifications drive and stabilize these different gene expression programs, and are essential for maintaining a persistent infection in dividing cells. How these epigenetic controls are coordinated with host cell biology and environmental factors remains an important area of future research. Recent advances in deep-sequencing methods and genome-wide bioinformatics analyses have revealed new insights into the complex epigenetic regulation of viral and cellular genomes. These studies show a dynamic interplay of histone and DNA modifications, a complex patterning of transcription factor binding, nucleosome positioning, and chromatin boundaries, and a regulatory role for higher-order chromatin structures and host-chromosome attachments. Deciphering the epigenetic code of cellular and viral genomes will require a more complete understanding of the molecular “writers”, “readers”, and “erasers” and how they are orchestrated to function in a highly coordinated and integrated process. Identifying the epigenetic and systems features that control EBV infection and latency will undoubtedly reveal important new insights into the mechanisms that control viral persistence and pathogenesis, and may provide new therapeutic targets for the treatment of EBV-positive tumors and associated disease.

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Figure 1. Summary of EBV latency type gene expression and promoter utilization

A. The EBV double-stranded genome map showing the position of regulatory elements and the location of latent transcripts, including non-coding RNA. The coordinates correspond to the 172 kbp reference viral genome. **B.** (i) Early stage transcription during primary infection of B-lymphocytes. Wp initiated transcription of EBNA2 is observed prior to Cp utilization. Qp may be used for generation of EBNA1 transcripts also required for the switch to Cp and establishment of Type III latency. (ii) Type III is observed in LCLs and in B-cell lymphomas associated with immunosuppression. Type III latency utilizes the Cp to generate several alternatively spliced and multicistronic transcripts that generate EBNA-LP, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, and EBNA1. Type III latency also produces LMP1 and LMP2 transcripts from a convergent promoter region. Non-coding RNAs are also expressed in both latency types, but are not shown in this schematic. (iii) Type I latency is found in most Burkitt Lymphomas and is thought to occur during natural infection during B-cell maturation into memory B-cells. Type I latency utilizes Qp to generate EBNA1 only transcript.

Figure 2. A Chromatin Boundary Function of CTCF at Qp

CTCF binds upstream of the Qp TSS and is required for maintaining Qp activity in Type I latency. In Type III latency, Qp is repressed by EBNA1 binding to the TSS. In genomes where CTCF binding site is genetically disrupted at Qp, transcription initiation is gradually silenced by the invasion of H3K9me3 and DNA methylation. CTCF binding is also required for the activity of Qp in Type I and the stable binding of EBNA1 in Type III latency.

Figure 3. A DNA Looping Function of CTCF at OriP and Cp or Qp

(Left panel) During Type I latency OriP (orange) forms a loop with the active Qp (green arrows) that is mediated by CTCF-cohesin binding sites. (Right panel) During Type III latency OriP forms a loop with Cp that depends partly on an intervening CTCF binding site. We envision that DNA looping to OriP recruits transcriptional promoters to an active chromatin domain.

Figure 4. A Chromatin Hub Function of OriP

OriP can function as a transcriptional enhancer for Cp and LMP1p in Type III latency. OriP interacts with EBNA1, ORC, and SNF2h chromatin remodeling factors. OriP can form stable loops with Cp and LMP1 mediated by CTCF and cohesins that bind in close proximity to these target promoters forming a viral Locus Control Region (LCR). Elevated H3K4me3 at OriP provides an Active Chromatin Hub (ACH).

Table 1
Summary of EBV encoded genes that affect host cell epigenetic regulation

Viral Gene	Effect	Reference:
EBNA1	Altering DNA methylation	104
	Altering telomere functions	105
	Altering nucleosome phasing	111
EBNA2	Interacting with HAT and remodeling viral and host epigenome	91-93
	Interacting with hSNF5/In and remodeling nucleosomes	94
EBNA3C	Recruiting PRC and repressing host tumor suppressors	96
LMP1	Activating DNMT1 through JNK-AP-1 signaling	50, 101
LMP2A	Activating DNMT1 through STAT3 signaling	102
BART	Targeting cellular genes involved in host epigenetic programming	103