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Lying low-chromatin insulation in persistent DNA virus infection

Christy S Varghese, Joanna L Parish and Jack Ferguson

Persistent virus infections are achieved when the intricate balance of virus replication, host-cell division and successful immune evasion is met. The genomes of persistent DNA viruses are either maintained as extrachromosomal episomes or can integrate into the host genome. Common to both these strategies of persistence is the chromatinisation of viral DNA by cellular histones which, like host DNA, are subject to epigenetic modification. Epigenetic repression of viral genes required for lytic replication occurs, while genes required for latent or persistent infection are maintained in an active chromatin state. Viruses utilise host-cell chromatin insulators, which function to maintain epigenetic boundaries and enforce this strict transcriptional programme. Here, we review insulator protein function in virus transcription control, focussing on CCCTC-binding factor (CTCF) and cofactors. We describe CTCF-dependent activities in virus transcription regulation through epigenetic and promoter–enhancer insulation, three-dimensional chromatin looping and manipulation of transcript splicing.

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Introduction

Chromatin insulator proteins are DNA-binding proteins that function to maintain epigenetic boundaries and facilitate long-range chromatin interactions in the host genome [1]. They therefore play a defining role in the control of transcription. Multiple chromatin insulator proteins have been described, including the CCCTC-binding factor (CTCF).

CTCF is highly conserved and ubiquitously expressed in bilaterian metazoans and binds with high affinity to thousands of cell-type-specific as well as ubiquitous target sites [2]. DNA binding occurs via distinct combinations of the 11 zinc finger domains within the protein. Differential usage of zinc finger combinations likely explains the apparent sequence diversity in binding sites and the diverse functional consequences of CTCF binding [3], including epigenetic insulation, enhancer blocking, alternative splicing and formation of short- and long-range chromatin loops [4–6••]. Although CTCF can bind to DNA independently of other protein complexes, numerous studies have identified cofactors that either bind directly to CTCF or co-bind at specific genomic loci [7].

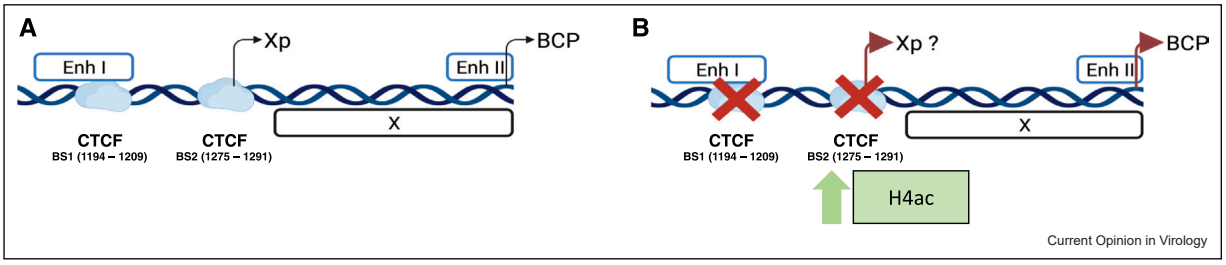
The most frequently co-bound protein complex is the cohesin complex, a tripartite protein ring consisting of four core subunits: SMC1, SMC3, SCC1/Rad21 and SCC3. This complex is able to modulate the transcription and insulator functions of CTCF by stabilising inter- and intrachromosomal interactions [6••,8]. Several other host proteins are enriched at CTCF-bound sites, including the transcriptional regulator Yin Yang 1 (YY1) [9] and poly-ADP ribosylase protein 1 (PARP1) [10].

For life-cycle completion, diverse viruses have evolved common mechanisms to interact with and manipulate the host-cell environment [11]. Several DNA viruses that establish persistent infection have been shown to utilise CTCF and associated cofactors to control virus transcription in both latent and lytic phases of infection. This review will highlight the commonalities and diversities of cellular insulator protein complex utilisation with a focus on four major virus families — hepadnaviruses, herpesviruses, papillomaviruses and retroviruses.

Hepadnaviruses

Hepatitis B virus (HBV) is a partially double-stranded small DNA virus with a genome size of approximately 3.2 kb and is the prototype member of the Hepadnaviridae family. HBV infection is the leading cause of liver disease with an estimated 250–260 million chronically infected individuals worldwide [12]. The development of chronic HBV infection is largely dependent on age at acquisition, with most perinatal cases leading to viral persistence [13]. Despite a deepening understanding of the HBV life cycle and host-factor utilisation, there are still important unanswered questions regarding the regulation of virus

Figure 1



CTCF recruitment to HBV cccDNA represses enhancer/promoter activity. **(a)** Two conserved CTCF-binding sites have been identified in HBV situated within enhancer-I and HBx promoter regions, respectively. **(b)** Abrogation of CTCF binding results in increased transcription of preC/pgRNA from the cccDNA template, coincident with increased deposition of the H4ac throughout the HBV genome.

episome persistence and transcription regulation in chronic HBV infection.

Following host-cell entry, the HBV genome is converted to a covalently closed circular DNA molecule (cccDNA), which persists in infected cells and serves as the transcriptional template. The HBV cccDNA contains two viral enhancer elements, Enhancer I and II and several transcriptional promoters that drive expression of four overlapping transcripts from the sense strand. Two highly conserved CTCF-binding sites have been identified within Enhancer I and the X ORF promoter (Xp) (Figure 1). Abrogation of CTCF binding at these sites results in an enrichment of activating epigenetic histone modifications and enhanced virus transcription, suggesting that CTCF functions to attenuate transcription from cccDNA in persistently infected cells [14•].

Interestingly, the CTCF cofactor YY1 has been shown to bind to the HBV basal core promoter, upstream of both viral enhancer elements. YY1 binding is enhanced by interferon alpha (IFN-α) resulting in repression of HBV transcription [15]. Whether CTCF and YY1 function cooperatively to repress HBV transcription is yet to be determined, but a recent study using a recombinant cccDNA cell model has demonstrated that YY1 and HBV X protein (HBx) form a protein–protein complex to mediate spatial regulation of cccDNA at a specific, highly active enhancer region on host chromatin [16•]. In contrast to the findings of Belloni et al. [15], Shen et al. [16•] demonstrate that depletion of YY1 resulted in reduced HBV transcription and conclude that this is due to disruption of cccDNA interaction with a strong enhancer element at chromosome 19p13.11. The exact role of YY1 in regulating HBV transcription is therefore not clear but likely to be context- and life-cycle-stage specific.

Herpesviruses

Herpesviruses are a family of large double-stranded DNA viruses with genomes of 125–240 kb in size. The herpesvirus family is subdivided based on cell tropism and genome organisation into ?-herpesviruses (herpes simplex virus (HSV) and varicella zoster virus), ?-herpesviruses (human cytomegalovirus (HCMV)) and ?-herpesviruses (Epstein–Barr virus and Kaposi sarcoma-associated herpesvirus (KSHV)) groupings. Herpesviruses have two very distinct replicative cycles that often occur in distinct cell types and tissues [17]. The initial site of infection usually supports lytic replication, resulting in fulminant production of infectious progeny. Establishment of persistent infection at a secondary anatomical site or cell type can facilitate viral latency, characterised by highly restricted virus gene expression in the absence of progeny virus production. Establishment of herpesvirus latency and reactivation is governed by epigenetic modulation of viral DNA and specific recruitment of host transcriptional regulators and insulators.

α-herpesvirus

Lytic replication of HSV at the site of primary infection causes oral herpes or genital herpes and is associated with rare but severe complications such as encephalitis. HSV latency is established in the nucleus of peripheral neurons after transport along the nerve axon to the ganglion. Periodic reactivation results in recurrent lesions at the primary site of infection. Upon initial infection, HSV DNA becomes rapidly chromatinised and silencing of virus transcription during latency establishment is induced by epigenetic silencing of the viral DNA, including marked enrichment of repressive trimethylation of lysine 27 of histone 3 (H3K27Me3) [18].

Several studies have demonstrated a key role for CTCF-mediated epigenetic insulation in HSV-1 latency [19–21]. Seven putative CTCF insulators have been described in latent HSV-1 genomes that flank the latency-associated transcript (LAT) and immediate–early (IE) gene regions [19]. Expression of LAT, encoded on the sense strand, is maintained by permissive histone modifications, yet the adjacent lytic-specific antisense

ICP0 gene is epigenetically silenced. The LAT intron contains an array of CTCF-binding sites that are bound by CTCF in latently infected cells and insulate the ICP0 promoter from LAT enhancer activation and prevent inappropriate activation of the IE lytic genes [20]. Reactivation of lytic replication in latently infected cells results in rapid disruption of CTCF binding within the LAT enhancer, which is thought to drive activation of IE gene expression via the LAT enhancer [22]. Consistent with these findings, siRNA-mediated depletion of CTCF in latently infected neurons results in reactivation of lytic replication characterised by long-term and persistent shedding of infectious virus [23]. Deletion of CTCF-binding sites within the LAT coding region reduces the ability of the virus to reactivate [24•], suggesting that CTCF binding is important for maintenance of latency and that eviction is an important step in virus reactivation.

CTCF-mediated transcriptional insulation appears to require co-binding of the cohesin complex at or near CTCF insulators. In latent HSV-1 genomes, cohesin is specifically enriched at four out of seven putative CTCF insulators. Given the role of cohesin in the stabilisation of chromatin loops, the authors conclude that co-binding of cohesin at insulator boundaries suggests that stabilised chromatin loops may be important in HSV-1 transcriptional latency [25••]. Interestingly, the CTCF insulator situated between the IE ICP4 open-reading frame and the LAT enhancer was devoid of the core cohesin complex protein, SCC1, suggesting that this particular CTCF-bound region blocks LAT enhancer activation of IE genes during latency via a linear rather than looping mechanism [25••]. It remains to be determined whether a switch in CTCF–cohesin-mediated chromatin looping plays a role in the latent-to-lytic switch of the viral life cycle.

CTCF and cohesin can also play a role in the lytic replication of HSV-1. CTCF and core cohesin subunits, SCC1 and SMC1, are enriched at lytic HSV-1 replication compartments and siRNA knockdown of either cohesin subunit results in reduced RNA polymerase-II occupancy and activity, and increased H3K27Me3 enrichment concomitant with reduced virus transcription, suggesting that cohesin plays a role in preventing chromatin silencing and latency establishment [26]. However, the location and mechanism of cohesin recruitment to HSV-1 genomes in lytic replication has not yet been determined.

β-Herpesvirus

HCMV infection is highly prevalent, and, like all herpesviruses, initial infection is followed by the establishment of lifelong latency [27]. HCMV latency is maintained via strict epigenetic repression of the major immediate–early promoter (MIEP) to prevent expression of lytic genes, while maintaining expression of a restricted set of genes required for latent virus replication. Repression of the MIEP during latency is in part controlled by CTCF recruitment to the MIEP enhancer element [28] and interestingly, CTCF recruitment has been shown to be dependent on the activity of the viral G-protein-coupled receptor, US28 [29•]. Whether CTCF strictly functions to insulate the MIEP from epigenetic activation during latency in a linear fashion or via a more complex stabilisation of chromatin loops remains to be determined.

γ-Herpesvirus

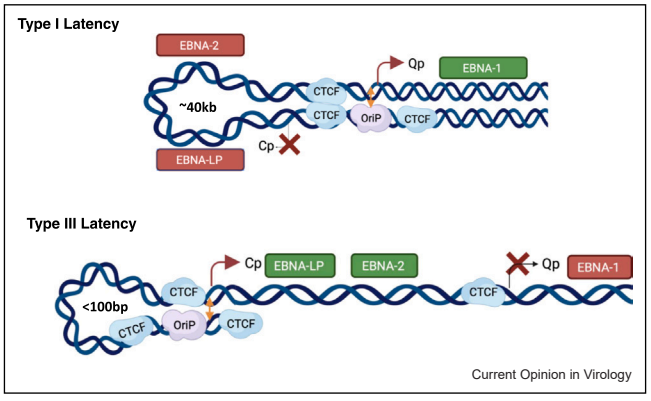
Persistent infection with KSHV is linked to Kaposi's sarcoma, primary effusion lymphoma, multicentric Castleman's disease and KSHV inflammatory cytokine syndrome [30]. At least 30 CTCF-binding sites have been identified in KSHV genome by ChIP-Seq, which are differentially bound during latent and lytic virus replication; induction of lytic replication results in reduced CTCF occupancy within broad regions of the viral genome, but binding at most sites in the latency gene locus is preserved [31]. The cohesin subunit SCC1 colocalises with twelve of the CTCF-bound regions in latent viral genomes and lytic reactivation results in a rapid loss of SCC1 in all but two regions. siRNA-mediated depletion of either SCC1 or CTCF results in enhanced transcription and lytic virus replication, suggesting that CTCF and SCC1 act to restrict lytic virus production [31].

A dominant CTCF/SCC1-binding cluster in the major latency-control region has been described. Interestingly, this binding cluster lies between the divergent latent and lytic promoters that drive expression of multicistronic transcripts consisting of key latency genes such as LANA (ORF73), vCyclin (ORF72) and vFLIP (ORF71), transcribed in the antisense orientation, and lytic-associated genes K14 and ORF74 (vGPCR), encoded in the sense orientation. Reactivation of lytic virus replication results in reduced CTCF/SCC1 binding to the major latency-control region and deletion of the CTCF-binding sites resulted in both SCC1 loss and increased lytic gene transcription [32]. A recent study reported that cohesin binding to KSHV genome is CTCF-dependent, whereas CTCF binding does not require cohesin [33•]. In line with these findings, siRNA-mediated depletion of SCC1, SMC1 and SMC3 induced KSHV lytic cycle transcription, but depletion of CTCF had no effect [34]. Notably, the CTCF-dependent insulator in the latency-control region has been shown to mediate a specific chromatin loop association between the KSHV latency-control region and the 5' promoter region of ORF50, which encodes the IE gene RTA, during latent infection, which is disrupted upon lytic reactivation [35]. These findings show that dynamic

CTCF–cohesin interactions coordinate lytic and latent gene control in KSHV.

EBV infection commonly causes acute infectious mononucleosis, but has also been shown to be a causative factor in a range of solid and blood cancers, including Burkitt lymphoma, nasopharyngeal carcinoma, gastric carcinoma, Hodgkin lymphoma and some T-cell lymphomas [36]. The EBV genome is maintained as a chromatinised episome and can adopt at least four latency types and associated transcriptional repertoires that can be aligned with the diverse clinical outcomes [37,38]. Latency-type 0 is established in non-dividing B cells and characterised by an absence of virus transcription and type-I latency is associated with Qp promoter-driven EBNA-1 mRNA expression only. Type-II latency is associated with LMP1 and LMP-2 expression alongside EBNA-1, and type-III latency is characterised by activation of the Cp promoter driving expression of EBNA-2 and -LP in addition to EBNA-1, LMP1 and LMP-2. CTCF plays a key role in the establishment of specific latency types. In type-I latency, CTCF binding between the OriP enhancer and Cp promoter represses Cp-driven transcription, including EBNA-2 [39] and binding upstream of the Qp promoter is essential for epigenetic insulation of Qp and maintenance of EBNA-1 expression [40]. CTCF binding to the same regions upstream of the Cp and Qp promoters is also observed in type-III latency. However, analysis of specific long-range chromatin interactions within EBV episomes has revealed that CTCF mediates chromatin looping between the OriP enhancer and Qp promoter in type-I latency and the Cp promoter in type-III latency I [41], and global analysis of 3D episome structure has revealed that a distinct topological arrangement is associated with different latency types [42••] (Figure 2). The CTCF-dependent establishment of these alternative stable chromatin conformations is an important regulator of latency-type establishment. The DNA-binding capacity and insulator function of CTCF is in part regulated by covalent post-translational modifications, including poly-ADP ribosylation catalysed by PARP1 [43]. PARP1 has been shown to colocalise and stabilise CTCF and cohesin binding to the EBV genome regulating latency-type maintenance and restricting lytic reactivation of EBV [44]. The differential chromatin looping observed in type-I and type-III latency appears to be regulated by PARP1 enzyme activity; chemical inhibition of PARP1 results in major 3-dimensional rearrangement of EBV episomes and transcription dysregulation [42••].

Figure 2



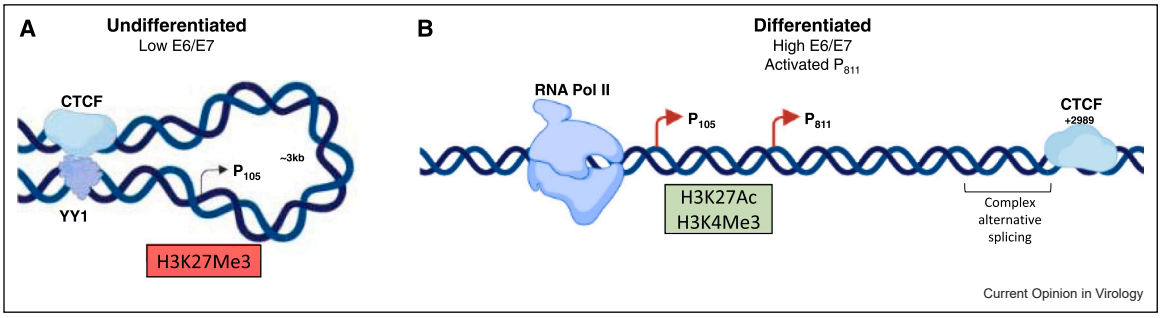
CTCF-dependent regulation of type-I and -III latency of EBV. **(a)** Type-I latency occurs when Cp is silenced through DNA methylation. CTCF binding between the OriP enhancer and Cp promoter represses Cp-driven transcription and binding upstream of the Qp promoter is essential for epigenetic insulation of Qp and maintenance of EBNA-1 expression. Here, a chromatin loop of ~40 kb is stabilised by intermolecular CTCF interactions between OriP and Qp, resulting in silencing of Cp and EBNA-2 and EBNA-LP expression while maintaining activation of Qp driving EBNA-1 expression. **(b)** The type-III latency transcriptional programme is co-ordinated via CTCF stabilisation of chromatin interactions between the Cp promoter and OriP (immediately upstream). This drives activation of the Cp promoter and EBNA-LP/EBNA-2 expression and prevents activation of the Qp promoter.

Papillomaviruses

Human papillomaviruses (HPVs) are a large family of viruses with small double-stranded DNA genomes of approximately 8 kb. All HPVs infect keratinocytes within cutaneous and mucosal epithelia and infection with the majority of these viruses is either asymptomatic or results in benign warts that are effectively cleared by the host. However, thirteen HPV types, including HPV-16, -18 and -31, are defined as high-risk due to their causal association with cancers of the oropharyngeal and anogenital tracts [45]. Infection with these high-risk HPV types can result in the establishment of persistent infection, which is a risk factor for cancer development. While the HPV life cycle does not include a bone fide latent transcriptional programme, models of persistent infection have shown that epigenetic and host transcription-factor repression of the chromatinised viral episome restricts gene expression [46–48]. Similarly, HPV gene expression is epigenetically restricted in models of early disease with sequential epigenetic activation of virus oncogene expression during disease progression [49]. Whether truly latent oncogenic HPV infection is achieved in vivo is unclear, but analysis of non-cancerous cervical tissue in women aged between 35 and 79 years revealed a surprisingly high prevalence of high-risk HPV DNA (42%), often in the absence of any detectable virus transcription, suggesting latent persistent infection [50].

Epigenetic regulation of HPV18 transcription is in part regulated by recruitment of CTCF to a conserved site within the E2 ORF of the early gene region [51]. In

Figure 3



CTCF function in chromatin looping and epigenetic transcription regulation of oncogenic HPV. **(a)** HPV gene expression is tightly controlled in undifferentiated basal cells and the expression of viral early genes (E6, E7) is attenuated due to the formation of a chromatin loop on the HPV18 viral genome between CTCF and its cofactor, YY1. **(b)** Upon terminal cellular differentiation, YY1 protein expression is reduced, resulting in release of the repressive chromatin loop and loss of PRC complex recruitment. Derepression and increased accessibility of the viral enhancer results in increased RNA polymerase-II recruitment coincident with H3K4Me3 deposition, resulting in enhanced E6/E7 oncogene expression and late promoter (P_{811} in HPV18) activation.

contrast to HBV and the herpesviruses described above, CTCF binding to the HPV genome occurs downstream of the viral enhancer and transcriptional promoters. When bound to the HPV episome, CTCF functions to attenuate activity of the upstream viral promoters by co-ordinating the formation of a chromatin loop between the CTCF-binding site in the early gene region and the upstream viral enhancer. This chromatin loop is stabilised by interaction with the host transcriptional regulator YY1 that binds to an array of sites within the viral enhancer to repress virus transcription [52] (Figure 3). The CTCF–YY1-stabilised chromatin loop induces epigenetic repression of the viral episome that is notably disrupted upon cellular differentiation-induced activation of virus transcription and necessary of activation of the late viral promoter and differentiation-dependent expression of capsid genes [47•,53•].

In HPV31, CTCF has been shown to be enriched in the late gene-encoding region, which contains three consensus CTCF-binding sites. Here, CTCF recruitment is important for co-binding of cohesin, and while the functional consequences of CTCF and cohesin recruitment to the late gene region in transcription regulation and early life-cycle events are not clear, recruitment and phosphorylation of the cohesin subunit SMC1 does appear to be important for cellular differentiation-induced viral genome amplification [54].

Retroviruses

It is estimated that ~15 million people worldwide are chronically infected with human T-cell leukaemia virus (HTLV-1), a causative agent of adult T-cell leukaemia [55]. The HTLV-1 genome is comprised of two copies of single-stranded RNA that is reverse-transcribed into double-stranded proviral DNA and integrates into transcriptionally active regions of the host genome as single copy. The proviral DNA serves as a template for gene transcription that occurs on both the plus- and minus-DNA strands upon initial infection. However, chronic infection is associated with CpG dinucleotide methylation and epigenetic silencing of plus-strand transcription, while minus-strand transcription is constitutively active driving expression of HTLV-1 bZIP factor, required for suppression of plus-strand transcription (reviewed by [56]). A CTCF-binding site has been identified within the pX region of the HTLV-1 genome, adjacent to the 3' untranslated region (UTR, which is important for the maintenance of epigenetic insulation of the proviral DNA, most likely to maintain minus-strand transcription) [57]. CTCF recruitment to the pX-binding site was also important for insulation of viral enhancers, thereby regulating inappropriate virus transcription, and effects on HTLV-1 mRNA splicing were observed when CTCF binding was abrogated [57]. Notably, integration of HTLV-1 proviral DNA into the host genome has been shown to have profound consequences for host chromatin structure and gene expression, effectively through the insertion of an ectopic CTCF-binding site, contributing to insertional mutagenesis [57–59]. Local changes to host transcription by proviral insertion, including upregulation of upstream antisense expression in all T-cell clones analysed, have been demonstrated. Long-range alteration of host transcription of specific genes encoded on the same chromosome as the viral-integration site was also observed. These changes to host-cell transcription are driven by an alteration of chromatin structure by the creation or disruption of CTCF-dependent chromatin loops within the infected chromosome [58••]. While each T-cell clone appears to have a unique fingerprint of chromatin rearrangements,

it is hypothesised that those events that provide a selective advantage of T-cell clones may contribute to carcinogenesis.

Conclusions

Host insulator protein complexes are commonly hijacked by diverse DNA viruses to aid chronic or latent persistence. The utilisation of the most widely studied chromatin insulator, CTCF, has revealed that for the most part, CTCF functions to attenuate lytic virus expression and contributes to evasion of host immune clearance. In-depth analysis of how specific virus families utilise CTCF to fine-tune virus transcription in specific cell types and virus life-cycle stages has revealed intricate mechanisms of CTCF-dependent regulation of viral episome topology and epigenetic status in the control of latent and lytic replication cycles and viral persistence. These studies have not only revealed novel strategies of transcription regulation, common between diverse virus families, but have begun to highlight the importance of alterations on chromatin structure and host transcription insulation in virus-driven host chromatin disruption during carcinogenesis.

Conflict of interest statement

None.

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