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The latency-associated nuclear antigen, a multifunctional protein central to Kaposi's sarcoma-associated herpesvirus latency

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Abstract

Latency-associated nuclear antigen (LANA) is encoded by the Kaposi's sarcoma (KS)-associated herpesvirus (KSHV) open reading frame 73. LANA is expressed during latent KSHV infection of cells, including tumor cells, such as primary effusion lymphoma, KS and multicentric Castleman's disease. Latently infected cells have multiple extrachromosomal copies of covalently closed circular KSHV genomes (episomes) that are stably maintained in proliferating cells. LANA's best characterized function is that of mediating episome persistence. It does so by binding terminal repeat sequences to the chromosomal matrix, thus ensuring episome replication with each cell division and efficient DNA segregation to daughter nuclei after mitosis. To achieve these functions, LANA associates with different host cell proteins, including chromatin-associated proteins and proteins involved in DNA replication. In addition to episome maintenance, LANA has transcriptional regulatory effects and affects cell growth. LANA exerts these functions through interactions with different cell proteins.

Keywords

AIDS; HIV; human herpesvirus 8; Kaposi's sarcoma; lymphoma; viral latency

Kaposi's sarcoma-associated herpesvirus

Kaposi's sarcoma (KS)-associated herpesvirus (KSHV) or human herpesvirus 8, a γ-2 herpesvirus, is the most recently discovered human herpesvirus [1,2]. Viruses related to KSHV (other γ-2 herpesviruses) are found in many mammalian species (e.g., herpesvirus saimiri [HVS; New World monkeys], murine γ herpesvirus 68 [rodents] and rhesus rhadinovirus [Old World monkeys]) suggesting that these viruses are ancient and have co-evolved with their hosts (for reviews see [3,4]). The KSHV genome is co-linear with other γ-2 herpesviruses, and similar to the human γ-1 herpesvirus, Epstein–Barr Virus (EBV). These viruses share a common genomic organization and blocks of genes have analogous roles in the viral life cycle. KSHV encodes approximately 75 genes, of which some are

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novel and some are homologs of known cellular genes. Importantly, KSHV and EBV are important tumorigenic agents in immunosuppressed individuals, such as transplant recipients and HIV/AIDS patients.

KSHV is tightly associated with KS, primary effusion lymphoma (PEL) and multicentric Castleman's disease (an aggressive lymphoproliferative disorder). KS remains the most prevalent tumor associated with HIV/AIDS patients [5]. Cell-mediated immunity plays a role in controlling the disease. Notably, KS lesions can regress with immune reconstitution by reducing immunosuppressive therapy in transplant recipients or by reconstitution of the immune system with HAART in AIDS patients [6–9].

A particularly aggressive form of KS occurs in African children and accounts for approximately 2–10% of all cancers in children in eastern and southern Africa [10–12]. KSHV seropositivity precedes KS and KSHV is found in all KS lesions in the presence or absence of HIV co-infection [8,13]. The seroprevalence of KSHV varies from approximately 50% of men who have sex with men to approximately 5% in the general population of the USA to 30–80% in endemic regions (for epidemiological studies see [8,11,13–18]). Serological assays are dependent upon the detection of different KSHV antigens, often including the protein encoded by open reading frame (ORF) 73, termed latency-associated nuclear antigen (LANA).

LANA is one of only a few KSHV-encoded proteins expressed during latency, which is the primary form of infection in both the normal host and in tumors [8,19,20]. LANA is approximately 1162 amino acids in length. A large repetitive region of acidic and glutamine-rich repeats comprise the middle of the protein separating the LANA N- and C-terminal regions (Figure 1). Heterogeneity in the length of the internal repeat region of LANA has been noted between KSHV isolates [21]. Some orthologs of LANA do not contain the internal repeats (e.g., murine γ-herpesvirus 68) (Figure 2), whereas other LANA orthologs, such as that of HVS, lack a proline-rich region [21–23]. The LANA ortholog of retroperitoneal fibromatosis herpesvirus, the homolog of KSHV that infects the pig tailed macaque, contains both a proline rich region and central acidic repeats, similar to LANA [24–26]. The N-terminal region of LANA contains a proline-rich region and associates with many chromatin associated proteins (Table 1). LANA amino acids 5–13 encode a chromosome binding motif that binds histones H2A/H2B [27]. The C-terminal region of LANA contains a unique leucine-rich domain and another repeat region, followed by unique sequence and associates with a number of host cell proteins and chromosomes (Table 1). The unique C-terminal LANA region self-associates to bind DNA and recognizes a specific DNA sequence within the terminal repeat (TR) region of KSHV. Many functions have been ascribed to the KSHV-encoded LANA, including episome persistence, transcriptional activity and growth effects on the cell.

LANA associates with chromosomes & KSHV TR DNA to maintain KSHV episomes

In tumors and latently infected cells, the KSHV genome (~200 kb) persists as a multiple copy (10–50 copies per cell), covalently closed circular extrachromosomal plasmid (episome). Only a small subset of KSHV genes are expressed in latent infection of PELs, including *LANA*, *v-FLIP* and *v-cyclin*, which are expressed from the same alternatively spliced transcript. Several viral miRNAs are also expressed in KSHV latently infected cells [28,29].

Episomes must overcome two major obstacles to persist in proliferating cells. Episomes must replicate prior to each cell division to avoid loss in copy number. In addition, episomes

must segregate to newly formed nuclei after mitosis to avoid being destroyed in the cytoplasm. LANA overcomes both of these obstacles by mediating replication of KSHV DNA, and segregating viral episomes to daughter nuclei.

During mitosis, LANA partitions replicated viral episomes to daughter cells. This is accomplished by LANA's ability to simultaneously bind host cell chromosomes and viral TR DNA sequences (Figure 3) [30–32]. Notably, the TR sequences account for approximately 25–30% of the KSHV genome, likely due to their role in episome persistence. The TR sequence of herpesviruses contains elements required for packaging the DNA into the nucleocapsid. Binding of a virally encoded gene product (LANA) to the TR to mediate episome persistence and replication is an additional and novel function for this element. Studies related to the epigenetic markers at the TR sequence may provide further insights into the relevance of this sequence in herpesviruses and into the role of host cell proteins that are recruited to it. Post-translational histone modifications provide scaffolds for protein assembly in many cellular processes including transcriptional activation and repression (chromatin remodeling), and DNA repair and replication. Histone modifications of heterochromatin, including H3K9me2, are found at the TRs of HVS [33]. Other histone marks implicated in transcriptional activation at the HVS TRs are dimethyl H3K4, and at both the HVS and KSHV TRs are H3K9ac [34,35]. Further evaluation of histone modifications at the TR would be of interest, as LANA-associated proteins include Hp1α and BRD4, which bind methylated and acetylated histones, respectively. Hence, the epigenetic modifications and the recruitment of cellular factors at the TR play a role in the life cycle of KSHV.

Chromatin-binding domains of LANA are essential for LANA-mediated episome persistence since mitotic chromosome binding is necessary for LANA tethering of episomes [30,31,35–38]. Both the N- and C-terminal regions of LANA bind chromatin (Figure 3). N-terminal LANA provides the dominant chromosome attachment region. Amino acids 1–32 of LANA diffusely distribute over chromatin and so LANA was speculated to interact with histones [31,38]. Further characterization of this interaction revealed that this N-terminal region of LANA binds to an acidic pocket at the interface of histones H2A and H2B [27,39]. Interestingly, this histone pocket also binds IL-33, suggesting that this may be a docking station that is underappreciated [40]. Full length LANA associates with chromosomes in the presence and absence of viral DNA sequences [41]. In the absence of episomes, the staining pattern of LANA exhibits broad staining of mitotic chromosomes, while in the presence of viral DNA LANA localizes to punctate foci at the sites of KSHV episomes [41]. This likely occurs as a result of C-terminal LANA binding to multiple high-affinity binding sites on KSHV TR DNA while simultaneously binding chromosomes.

Other DNA tumor viruses, including EBV and the papilloma viruses, encode nonhomologous genes with functions analogous to LANA [42,43]. For example, EBV has a similar system for episome segregation and replication fostered by the EBV encoded EBNA1 protein binding to the oriP DNA sequence of EBV [43–45]. The papilloma viruses utilize E1 and E2 proteins to facilitate episome maintenance and replication; E2 binds chromatin and tethers the genome to chromosomes [42,46–49]. Viral DNA and LANA colocalize on mitotic chromosomes [41]. However, in cells that are co-infected with EBV and KSHV, LANA and EBNA1 do not colocalize, suggesting differences in tethering mechanisms [50]. In mitotic cells, C-terminal LANA can localize to pericentromeric regions that do not associate with CenpA. This region of LANA is important for DNA binding and, similar to the N-terminal region of LANA, has a role in chromosome binding and episome persistence [37,51]. Colocalization of some LANA molecules with kinetechore-associated proteins CenpF and Bub1 has been noted [52]. EBNA1, however, does not associate with pericentromeric regions during mitosis but does appear as paired dots on sister chromatids,

which coincide with EBV episomes [53,54]. Depending on the type, HPV E2 staining appears to resemble LANA as pericentromeric or EBNA1 as paired dots [47,48]. Human HPV E2 has been shown to associate with the spindle apparatus during mitosis; however, LANA appears to depend on chromatin associations for partitioning to daughter cells. Therefore, episomal DNA viruses have evolved mechanisms to facilitate association with chromosomes to ensure that the virus is not lost during segregation of replicated chromosomes with each cell division.

The C-terminal domain of LANA is the most conserved region among the LANA orthologs (Figure 2 & 4) [26]. The TR DNA-binding domain of LANA is in the C-terminal region of the protein encompassing amino acids 996–1139 [51,55–58]. C-terminal LANA self associates and this self association is essential for DNA binding [56,59,60]. The structure of LANA binding to the TR has not been solved. However, the DNA-binding domains of EBV EBNA1 and HPV E2 have been crystallized bound to their respective DNA-recognition sequences [42,61,62]. Remarkably there is structural homology between the EBNA1 and E2 DNA-binding domains, despite the absence of sequence homology. Software modeling studies predict structural similarities between the DNA-binding domain of LANA and EBNA1, and by extension, E2, suggesting either evolution from a common ancestor or convergent evolution [32].

The C-terminal domain of LANA also associates with chromatin at pericentromeric regions, juxtaposed to CENPA, and occasionally at peritelomeric regions, and this association with chromatin assists in episome persistence [37,51]. Interestingly, a number of LANA-associated proteins localize with pericentromeric regions of chromosomes or are heterochromatin-associated proteins. These include MecP2, Sin3A, HP1α, ORC2, ORC6 and SUV39H1 [63–67], some of which associate with C-terminal LANA. It is unclear if LANA associates with these interacting proteins in a cell-cycle dependent manner given the varied functions of these LANA-associated proteins (Table 1).

The association of the pericentromeric protein, MecP2, and the HVS ORF73 is important for episome persistence. Knockdown of Mecp2 using siRNA inhibited HVS episome persistence [68]. MeCP2 is a DNA-binding protein that recognizes methylated cytosine residues and is implicated in both transcriptional repression and activation [69]. While the C-terminal region of HVS ORF73 (LANA homolog) associates with MeCP2, the N-terminal and C-terminal regions of KSHV LANA were found to associate with MeCP2 [63,66]. MeCP2 and HVS ORF73 associate with pericentromeric regions of chromosomes. Stuber *et al*. found that MecP2 was scattered from chromocenters in mouse cells expressing LANA [70], although Matsumura *et al*. found that MecP2 relocalized LANA to chromocenters in mouse cells [63,66]. It is curious that MeCP2 can associate with DAXX (an N-terminal KSHV LANA-associated protein, see Table 1) and ATRX complexes of proteins, which have recently been implicated in remodeling of chromatin by incorporation of histone H3.3 at pericentric and telomeric loci of mouse embryonic stem cells [71]. Therefore, many cellular processes may be affected by LANA and its binding partners in KSHV-infected cells.

There is strong evidence that LANA is required for episome maintenance. Firstly, cells stably expressing LANA can maintain TR-containing plasmids as an episome, whereas cells that do not express LANA cannot [30,32,41,55,58,72–74]. Knockdown of LANA using siRNAs or genetic deletion of LANA from a bacterial artificial chromosome (BAC) containing the viral genome leads to a loss of episomes [75–78]. Genetic deletion of ORF73, the LANA homolog, from the related murine γ-herpesvirus 68 viral BAC, alters the pathophysiology in infected mice [79–82]. The absence of ORF73 leads to a lack of viral spread and latency in the splenic compartment. In addition, Paden *et al*. demonstrated that

murine γ-herpesvirus 68 is integrated or linear in the absence of ORF73 in the B cell compartment, suggesting that LANA may be required for ligation and circularization of viral DNA, in addition to episome persistence [82]. Taken together, these data support the role of LANA in episome persistence.

The art of episomal maintenance and replication by viruses has many practical uses. For example, episomal vectors providing LANA or EBNA1 in *cis* or *trans* can be used for gene expression in the absence of integration [83]. This system, using EBNA1, has been used to express genes in fibroblasts to promote induced pluripotent stem cells, without the caveat of other expression systems that integrate into the host genome, indicating that episomal systems may prove useful for gene therapy [84]. Episomal systems have also been used for protein interaction studies [85]. Absence of LANA, and so absence of a mechanism for latency, may also prove useful in vaccine development [75].

LANA mediates DNA replication of KSHV episomes

LANA-mediated TR DNA replication is essential for episome persistence. In the absence of episome replication, KSHV DNA is rapidly lost from proliferating cells. Since only a few viral proteins are expressed in latent infection, and these do not include a viral-encoded DNA polymerase, host cell DNA replication machinery is used during latency to replicate episomal DNA. Cell replication machinery must assemble on the viral episome. Proteins associated with origins of DNA replication including ORC2, ORC3, ORC5 and MCM have been shown to be associate with glutathione-*S*-transferase-tagged fusion proteins of LANA or at the terminal repeat region by chromatin immuno-precipitation, suggesting that the terminal repeat region of KSHV may be an origin of replication [35,67,86]. Replication bubbles have been described for the EBV episome using 2D gel analyses or single molecule analysis of DNA, and the initially described origin of replication termed ‘oriP’ binds EBNA1. However, multiple origins of replication have now been described for EBV and KSHV, suggesting that multiple origins of replication may exist for DNA viral episomes [87–89]. Using Gardella gel analysis or the methyl-DNA sensitive restriction endonuclease DpnI and Southern blotting, it was shown that plasmids containing the TR sequence replicate in the presence of LANA but not in the absence of LANA [30,32,74,86,90]. Mutations in the N-terminal chromosome-associated region, encompassing amino acids 4–32 of LANA, indicate that these residues are critical for DNA replication as ascertained by DPNI sensitivity assays of TR-containing plasmids and Gardella gel analysis [36]. This could be related to priming the association of replication complexes, or for proper localization of the episome to replicate. Deletion of, or mutations within, amino acids 4–32 can greatly reduce or abolish episome persistence and replication as demonstrated by Gardella gel analyses and methylation sensitive restriction endonuclease digestion (DpnI) of TR-containing plasmids [36,90]. Similar to results described for EBNA1, the N-terminal region of LANA can be replaced by histone H1 and chromatin binding and replication of plasmids containing the terminal repeat are sustained [91,92].

C-terminal LANA binds TR DNA and this binding is essential for DNA replication and episome persistence. LANA binds specifically to a sequence within the TR [30,56,58,59,93]. LANA binds co-operatively to two adjacent binding sites located within each TR unit, a high-affinity site and a lower-affinity site. LANA binds to the high-affinity site with a kd of approximately 1.51 nm [59]. Near these two binding sites is a 32 bp GC-rich element and together they form the minimal LANA DNA replication element [55]. LANA binding to its TR-binding site is essential for DNA replication and episome persistence [56]. Similar to EBNA1, the LANA DNA binding domain located between residues 996–1139 must self associate to bind its recognition sequence [56,60]. Specific LANA residues important for TR binding within this region have been mapped by alanine substitution mutagenesis [51,94].

Using a biotinylated TR probe with the LANA recognition sequence, Hu *et al*, identified 30 proteins including SSRP1 [95]. SSRP1 is a member of the FACT complex, which is involved in histone–histone and histone–DNA interactions involving replication, transcription and DNA repair (for review see [96]). Another LANA-associated protein involved in latency, nucleophosmin (NPM), is also a part of the FACT complex [97]. RNAi mediated knockdown of SSRP1 diminished LANA-mediated replication of TR-containing plasmids, suggesting the FACT complex may be involved in LANA-mediated functions of replication. More studies regarding LANA and replication of KSHV episomes are required.

LANA & transcription

Several studies have examined gene expression levels by microarray analyses in KSHV-infected endothelial cells or hematopoietic cells. Infection with KSHV alters transcriptional signatures [98–106]. Other studies have focused on the effects of a single KSHV gene, such as LANA, on gene expression. Telomerase-immortalized endothelial cells, transduced with a retroviral vector containing LANA, were shown to repress approximately 80 genes as compared with empty vector control cells [107]. By chromatin immunoprecipitation, LANA was shown to bind to the promoters of some of the identified repressed genes including *CDH13*, *CREG* and *CCND2*. It was postulated that this repression was at the transcriptional level and due to the induction of methyltransferase activity recruitment by LANA. Cytosine methylation of CpG islands is felt to result in silencing of promoters, silencing of transposons, monoallelic expression of imprinted genes and X-chromosome inactivation (for reviews see [108–110]). CpG islands are found in promoters of many genes including some viral genes. Nuclear cytosine methyltransferase enzymes of eukaryotes include Dnmt1, Dnmt3a and Dnmt3b. Maintenance methylation is attributed to Dnmt1, which preferentially recognizes hemimethylated DNA during semiconservative replication [111,112]. Dnmt3a and DNMT3b have a role in the methylation of repeated sequences, such as transposons and pericentric repeats [113]. Histone-modifying enzymes and chromatin remodeling factors also associate with DNA methyltransferases. Dnmt3a, a *de novo* DNA methyltransferase, was increased in the chromatin-associated fraction in the presence of LANA [107]. Bacterially expressed glutathione-*S*-transferase-LANA and *in vitro* translated DNMTs were shown to interact and epitope-tagged Dnmt3a recruitment to the CCND2 promoter was enhanced in the presence of LANA. In addition, LANA was found to associate with DNMT3a by yeast two-hybrid analyses [63]. Methylation of the TGFβII receptor promoter element was also noted in the presence of LANA but not in the absence of LANA [114]. These data suggest that epigenetic factors, including DNA methylation and chromatin remodeling, may be involved in epigenetic reprogramming by LANA.

Transcriptional profiling of uninfected BJAB B lymphoma cells in the presence or absence of LANA indicates that LANA expression has an effect on transcription [102,115]. Several interferon responsive genes were induced approximately three-fold by LANA, including STAT1 and Staf-50. BJAB cells with a doxycycline inducible LANA were shown to affect the expression of approximately 186 genes [115]. Many of these genes are implicated in Rb/E2F-dependent pathways and WNT signaling. Several other studies demonstrate that LANA can induce or repress transcription using various promoter reporter constructs [102,107,115–120]. LANA was shown to activate its own promoter, SRE, SP1, ATF, CAAT and AP1 promoters, but repress an HIV LTR promoter. In terms of latency, LANA transactivates its own promoter [121]. LANA has also been shown to bind the KSHV lytic transactivator ORF50 (Rta), the ORF50 promoter, and to repress ORF50 expression as a mechanism to inhibit initiation of the viral lytic life cycle and so maintain latency [23,122,123].

LANA's association with the Mediator complex suggests that LANA can recruit the polII transcriptional machinery to activate transcription [117]. Consistent with this observation is

the association of LANA with transcriptional activators including CBP, CREB2, c-jun, KLIP, c-myc, Sp1, SRF, Stat3, Rb, Hif1α, KZLP and Tat (Table 1) [117,119,124–134]. Other transcriptional repressors like CIR, I-mfa, Sap30a, Sin3a and Jκ, also interact with LANA [63,64,134–136]. These interactions may have effects on viral persistence, transcription and growth transformation [63–65,137,138]. These data surrounding LANA's ability to repress or induce transcription may be at least partially dependent upon the cell line used, concentration-dependent effects of LANA expression and the diversity of reporters examined. Somewhat lacking is a clear connectivity between the number of reported transcriptional activators or repressors with which LANA associates and roles they play in the viral life cycle.

LANA & effects on cell growth

Control of cell cycle checkpoints and inhibition of apoptosis are hallmarks of proliferating tumor cells including PELs and KSHV-infected KS spindle cells. The association of LANA with many proteins involved in cell cycle regulation suggests that LANA promotes cell survival. For example, LANA prolongs the lifespan of HUVECS [139]. Data gathered from protein interaction studies support the role of LANA in cellular transformation as certain LANA-associated proteins can affect cell growth. For example, the retinoblastoma protein associates with LANA [133]. Several studies implicate LANA in activating E2F-dependent reporters or E2F-dependent genes [115,140]. LANA induces the expression of Id1 (inhibitor of DNA binding) at the mRNA and protein levels [118]. Since Id proteins have effects on cell cycle regulation, this may be one mechanism of LANA-mediated proliferation of KSHV-infected cells. Collectively, these data implicate LANA in increasing the replicative function of cells.

In addition, LANA associates with, and has effects on, the prototypical tumor suppressor p53 [79,138,141–144]. LANA inhibits p53-dependent transcription and apoptosis. Significantly, mutations in the p53 gene were not found in primary KS samples and functional p53 activity is detected in PEL cell lines [145–148]. The presence of wild-type p53 in KSHV-infected PEL cells suggested that small molecule inhibitors of p53–MDM2 interactions, like nutlin3a, may affect PEL growth. Using gel filtration analyses to examine molecular complexes, nutlin3a interfered with the formation of MDM2–p53–LANA complexes. Nutlin3a induced p53-dependent gene transcription, and caused apoptosis of PEL cells [149,150]. These findings demonstrate that LANA modulates p53-dependent pathways to prevent cell cycle arrest and apoptosis.

Many cellular processes associated with prolonged cellular survival are affected by LANA. Expression of LANA in mice can result in lymphoma [151]. The LANA promoter was found to function in $CD19^+$ B cells but not $CD3^+$ T cells in the spleen and bone marrow of transgenic mice [152]. Further studies revealed that LANA enhanced B cell responses to antigen in mice expressing the LANA transgene in B cells [153]. Prolonged cellular proliferation is associated with telomerase expression and LANA is an activator of telomerase reverse transcriptase expression [119]. LANA affects the stabilization of the *c-myc* oncogene by reducing the level of phosphorylation at T58 of c-myc, and protecting the phosphoylation of c-myc at S62. This LANA function promotes c-myc transcriptional activity and growth transformation properties [125,130]. Other signaling pathways associated with cancer, such as Notch and WNT pathways, are affected by LANA. LANA influences WNT signaling by nuclear trapping of GSK3β and stabilizing β-catenin [154–157], although this mechanism was questioned in one report [158]. LANA's effects on cell growth may be mediated by increases in survivin expression, an inhibitor of apoptosis [159]. RNAi-mediated knockdown of survivin affects the growth rate of KSHV-infected cells. LANA also associates with the oncogenes *Pim1* and *Pim3* [160]. These oncogenes are

elevated in LANA-expressing cells. LANA activates the Pim1 promoter, and LANA is phosphorylated by Pim kinases [160,161]. Proliferation of LANA-expressing cells and control cells was downmodulated in cells expressing shRNAs to Pim1, suggesting that Pim1 can have affects on cell growth in the presence of LANA. However, Pim1 and Pim3 were found to associate with LANA in the lytic cycle and not in latent cells, implicating a complex role of these kinases in posttranslational modifications of LANA and in the viral life cycle [160]. LANA may stabilize HIF1α [126,142]. In so doing, LANA likely contributes to the Warburg effect, shifting the profile of metabolic pathways upon which KSHV-infected proliferating cells depend [162].

LANA has also been described to function as a component of the EC_5S E3 ubiquitin ligase complex using unconventional suppressor of cytokine signaling box-like motifs to target p53 and von Hippel–Lindau for degradation, which leads to a favorable environment for cell growth [163]. LANA's effects on p53 and von Hippel-Lindau protein stability have been questioned [158]. Notably, murine γ-herpesvirus 68 ORF73 has also been described to assemble an EC_5S E3 ubiquitin ligase to regulate NF-κb [164].

BRD4 associates with LANA and the murine γ-herpesvirus 68 ORF73 [165,166]. Bromodomain proteins have conserved structures that recognize acetylated histones or acetylated proteins. BRD proteins have roles in cell cycle regulation and certain cancers exhibit fusion proteins (translocations) with BRD family members [116,167–172]. LANA represses BRD4-induced activation of the cyclin E promoter [116]. Interestingly, the EBNA1 gene of EBV and the E2 protein of HPV also associate with BRD4, in part to mediate chromosome association and episome persistence (E2) and transcriptional activation (EBNA1) [42,49,120,173–175]. In fact, a BRD4 peptide fused to tat, to promote nuclear localization, inhibits BRD4 and HPV E2 association and ablates chromosome association. Mutations in E2 that inhibit association with BRD4 also inhibit transcriptional activation [42,176]. Taken together, these data suggest a role for BRD4 in the life cycle of at least some DNA viruses with mechanisms of transcription, chromatin association, and episome persistence.

Conclusion

In summary, LANA's functions are diverse within the viral life cycle of KSHV. LANA's best characterized functions include its critical role in the maintenance of latency, episome replication and episome persistence. LANA serves as a hub for many host cell interacting proteins. These associated proteins have functions in DNA replication, transcriptional regulation and growth control, and lend insight into the active and multifunctional role of LANA in many cellular processes. Understanding LANA's role in modifying or adapting host cell protein function will lead to a better understanding of viral latency and oncogenesis.

Future perspective

Viral proteins like LANA, involved in episomal maintenance and replication, are intriguing from an evolutionary perspective. The related γ-2 herpesviruses demonstrate species specificity, with some divergence of virus sequence and gene repertoire relating to their hosts, as well as differences in pathology. As seen with KSHV and other viral-driven tumors, during times of immunosuppression, progression of viral associated pathology, including malignancy, can occur. Understanding critical host cell pathways that are deregulated during immunosuppression that lead to KSHV tumors should reveal critical immune components for viral-mediated tumor suppression. Understanding how LANA deregulates many host cell proteins may also lead to a better understanding of cellular

transcriptional and growth control processes. Most importantly, since LANA is central to KSHV latency, it serves as an opportune target to prevent and treat KSHV malignancies.

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Executive summary

Latency-associated nuclear antigen mediates Kaposi's sarcoma-associated herpesvirus episome persistence

- By binding to chromosomes and Kaposi's sarcoma-associated herpesvirus (KSHV) terminal repeats, latency-associated nuclear antigen (LANA) bridges KSHV episomes to chromatin, ensuring proper segregation of episomes to daughter cells.
- By binding terminal repeat DNA and interacting with components of the replication machinery, LANA assists in the replication of KSHV episomes.

Two LANA regions associate with chromosomes

- LANA N-terminal and C-terminal regions associate with chromosomes.
- The N-terminal region of LANA docks in the histone H2A/H2B pocket and is essential for episome persistence.

LANA is a DNA-binding protein & binds KSHV terminal repeat sequence

- LANA self-associates to bind specific DNA sequences in the GC-rich KSHV terminal repeat.

LANA associates with host cell proteins to modulate transcription, chromatin remodeling & cell growth

- LANA promotes both transcriptional silencing and gene expression in different contexts.
- LANA has effects on cell growth by inhibiting apoptosis and promoting proliferation.

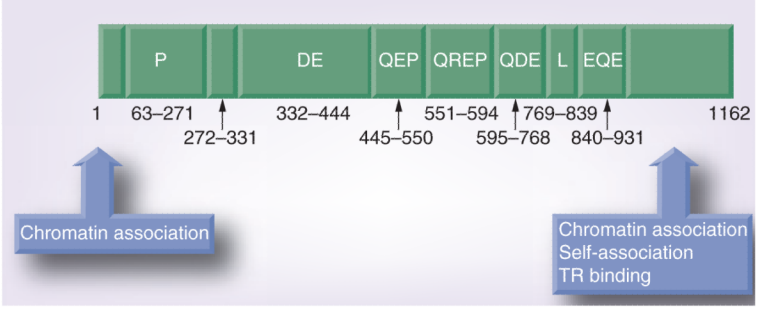

Figure 1. Latency-associated nuclear antigen protein

LANA consists of 1162 amino acids. Repetitive blocks of amino acids are noted (DE, QEP, QREP, QDE and EQE). Residues 63–271 contain a proline-rich region. Residues 769–839 contain a leucine-rich repeat region.

D: Aspartic acid; E: Glutamic acid; L: Leucine; P: Proline; Q: Glutamine; R: Arginine.

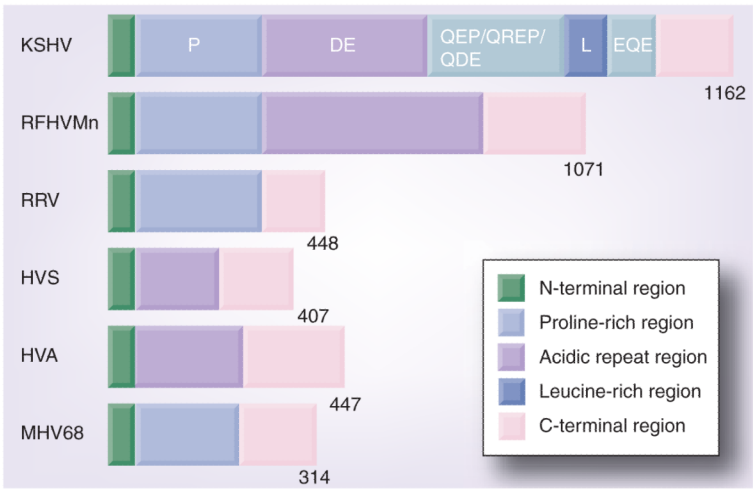

Figure 2. Schematic of latency-associated nuclear antigen and the ORF73 homologs

Similar regions between homologs of ORF73s from different γ-2 herpesviruses are indicated and compared with KSHV LANA. KSHV LANA (*Homo sapiens*); (NP_572129), HVS (squirrel monkey; NP_040275), HVA (spider monkey; NP_048045), RRV (rhesus macaque; AAD21406), MHV68 (vole; NP_044913), RFHVMn (rhesus pig tailed macaque; ABH07415).

Adapted from [26].

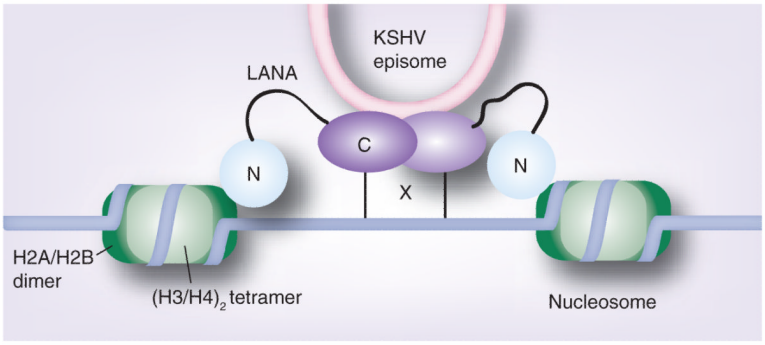

Figure 3. Model of latency-associated nuclear antigen binding to Kaposi's sarcoma-associated herpesvirus episome and cellular chromosomes

The C-terminal region of LANA (C) mediates self-association, binds to KSHV terminal repeat DNA in the KSHV episome, and binds to a putative protein (X) that associates with DNA (light gray line). The N-terminal domain of LANA (N) binds to core histones H2A/H2B, which are part of nucleosomes that also contain a H3/H4 histone tetramer.

KSHV: Kaposi's sarcoma-associated herpesvirus; LANA: Latency-associated nuclear antigen.

Adapted from [51].

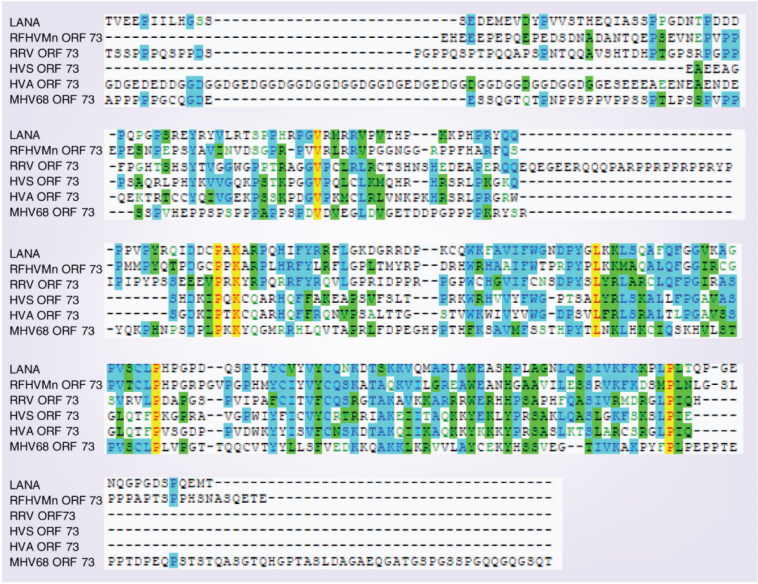

Figure 4. Amino acid alignment of the C-terminal regions of latency-associated nuclear antigen (aa933–1162) and ORF73 homologs

Yellow shaded regions indicate conserved amino acids; green shaded amino acids indicate conserved basic, acidic or hydrophobic residues; blue shaded regions Indicate conserved residues between two or more ORF73 homologs. Alignment was made using Vector NTi (Invitrogen, CA, USA).

Table 1

Latency-associated nuclear antigen-associated proteins.

Interactor	Name	LANA binding site†	Ref.
Transcription factors or chromatin-associated factors			
BRD4	Bromodomain protein 4	1133–1143	[116,166]
CIR	CBF1 interacting co-repressor	1–340	[64]
CBP/p300	cAMP response element binding protein	340–431, 950–1162	[128]
CREB2	cAMP response element binding protein	769–839	[129]
DAXX	Death domain-associated protein	320–344	[177]
Dek	DEcoRVK	986–1043	[63]
H1	Histone 1	1–1162	[31]
H2a/H2b	Histones 2a and 2b	5–13	[27]
Hiflα	Hypoxia inducible factor 1α	46–145	[126]
Hp1α	Heterochromatic protein 1α	1047–1062	[65]
I-mfα	Inhibitor of MyoD family α	995–1162	[135]
Jκ	Immunoglobulin Jκ region recombination signal binding protein	990–1162	[136]
c-jun	Oncogene 17	1–1162	[124]
KLIP	KSHV LANA interacting protein	1–317	[132]
KZLP	KRAB zinc finger binding protein	1–1162	[134]
MecP2	Methyl cytosine binding protein 2	1–15, 936–1162	[63,66]
Med25	Mediator 25	1–340	[117]
c-myc	Avian myelocytomatosis vial oncogene homolog	1–1162	[125,130]
p53	p53 tumor suppressor	441–1162	[143]
Rb	Retinoblastoma protein	803–990	[133]
Rta	Replication and transcription activator	990–1162	[123]
Sap30α	Sin3α-associated polypeptide	1–340	[64]
Sin3a	Paired amphipathic helix protein Sin3a	1–340	[64]
Sp1	Specificity protein 1	1–1162	[119]
srf	Serum response factor	1–1162	[117]
Stat3	Signal transducer and activator of transcription 3	933–1162	[131]
tat	Transactivator protein	762–1162	[127]
Replication			
NPM	Nucleophosmin	1–1162	[97]
Orc1	Origin recognition complex 1	1–340, 762–1162, 1001–1068	[86,178]
Orc2	Origin recognition complex 2	762–1162, 1001–1068	[86,178]
Orc3, 4 & 6	Origin recognition complex 3, 4 & 6	762–1162	[86,178]
Orc5	Origin recognition complex 5	1–340, 762–1162	[86,178]
SSRP1	Structure-specific recognition protein 1	1–1162	[95]
Kinases			
Gsk3β	Glycogen synthase kinase 3β	241–275, 1133–1147	[154,158]

Interactor	Name	LANA binding site†	Ref.
Pim1	Proviral integration site MuLV	762–1162	[160]
Pim 3	Proviral integration site MuLV	1–1162	[160]
Ubiquitin related			
Cul5	Culin 5	1085–1100	[163]
Elongin C	Elongin C	212–222	[163]
FBW7/Sel10	F box WD40 domain protein 7	1052–1082	[179]
VHL	Von Hippel–Lindau tumor suppressor	1–327	[163]
Enzymes			
DNMT1	DNA methyltransferase 1	1–340	[107]
DNMT3A	DNA methyltransferase 3A	1–15	[107]
DNMT3B	DNA methyltransferase 3B	1–340	[107]
Suv39h1	Suppressor of varigation 39h1	275–467	[180]
Ung2	Uracyl DNA glycosylase 2	762–1162	[178]
Miscellaneous proteins			
Bub1	Budding unhibited by benzimidazoles	1–340, 842–1162	[52]
CenpF	Centromere protein F	1–340, 842–1162	[52]
MNDA	Myeloid cell nuclear differentiation antigen	22–274	[181]
Numa	Nuclear mitotic apparatus protein	762–1162	[182]

†Numbers refer to amino acids of LANA (Genbank U75698) that have been mapped to interact with the stated protein. LANA: Latency-associated nuclear antigen.