Chapter 20
KSHV Latent Genes and Their Regulation

Dirk P. Dittmer

"Herpesviridae omnia divisa est in partes tres."

Herpesviridae are divided into three groups: alpha herpesviruses, beta herpesviruses and gamma herpesviruses. Only the two gamma herpesviruses, Kaposi sarcoma-associated herpesvirus (KSHV, or human herpesvirus 8) and Epstein–Barr virus (EBV, or human herpesvirus 4) are associated with human cancer. Herpesvirus lytic replication is also customarily divided into three phases: alpha or immediate early (IE), beta or early (E) and gamma or late (L). We base this classification on the temporal order of viral gene expression. Here, I propose that KSHV latent genes likewise may be divided into three categories based upon their pattern of transcription in KSHV-associated diseases (Table 20.1).

KSHV is associated with three proliferative malignancies in immune-compromised patients: Kaposi sarcoma (KS), primary effusion lymphoma (PEL) and the plasmablastic variant of multicentric Castleman disease (MCD) (reviewed in (Antman and Chang 2000; Ablashi et al. 2002; Dourmishev et al. 2003)). Overwhelming epidemiological evidence shows that KSHV infection is required for the disease phenotypes. Every tumor cell carries the viral genome and expresses KSHV latent proteins. Every cell that stably maintains the KSHV genome must express the latency-associated nuclear antigen (LANA), as this protein is required for latent episome maintenance (see Chapter 19). LANA is expressed in KS as well as in PEL and MCD (Kedes et al. 1997a; Kellam et al. 1997a; Dittmer et al. 1998; Dupin et al. 1999a). LANA transcription is regulated by the LANA promoter, which is constitutively active in all cell types. LANA-2/vIRF-3/K10.2 is only expressed in PEL, a tumor of B cell origin. By contrast K9/vIRF-1 is seen more prominently in KS, a tumor of endothelial origin. Another KSHV latent gene *Kaposin*/k12/t0.7 is also constitutively transcribed in all

D.P. Dittmer (✉)
University of North Carolina at Chapel Hill, Department of Microbiology and Immunology and Lineberger Comprehensive Cancer Center
e-mail: ddittmer@med.unc.edu

B. Damania, J. Pipas (eds.), *DNA Tumor Viruses*, DOI 10.1007/978-0-387-68945-6_20,

Table 20.1 The three categories of KSHV latency

	KS (in situ)	PEL, MCD (in situ)	TPA inducible	Genes
Type I	100%	100%	Minimal	LANA*, vFLIP*, vCYC*
Type II	0%	100%	Not inducible	LANA-2/vIRF-3
Type III	100%	100%	Maximal	*Kaposin**, K9/vIRF-1*

*A second, proximal promoter drives transcription in response to Rta/orf50.

KSHV-associated tumors. K9/vIRF-1 and *Kaposin* are greatly induced by phorbol ester (TPA) stimulation of PEL. The transcriptional response to TPA of LANA/orf73, vCYC/orf72 and vFLIP/orf71/K13, which are derived from differentially spliced, overlapping transcripts, is complex and LANA-2/vIRF-3/K10.5-10.6/K10.7 mRNA levels are impervious to TPA.

In sum, KSHV latent transcription seems divinely designed, or rather evolutionarily tuned, to respond to different host environments. This ensures commensal coexistence of virus and host under normal circumstances, but can lead to tumorigenesis in the setting of immune deficiency.

20.1 Profiling KSHV Transcription in Experimental Models and Primary Tumors

Herpesvirus transcription is divided into four stages: the three lytic stages (alpha, beta and gamma) and the latent stage. In KSHV, and in the related EBV, latency can be further divided into additional types, based on gene expression and host cell origin. Many excellent studies have profiled KSHV transcription genome-wide (Table 20.2). Although the KSHV transcript map is of yet incomplete (see Chapter 19 for the current transcript map), all gene expression profiling studies concur as to the identity of the KSHV latent genes (Fig. 20.1). These are LANA (orf73), vCYC (orf72), vFLIP (orf71), the viral miR cluster, *Kaposin* (orf K12) and LANA-2/vIRF-3. Except for LANA-2/vIRF-3, all other latent genes are clustered in the far right region of the viral genome. Except for LANA-2/vIRF-3, which is B-cell specific, all other latent genes are expressed in all KSHV-associated cancers and all experimental models of KSHV latency.

The kinetics of KSHV latent transcripts outside of long-term latency forms a focus of recent research. Chandran and colleagues found that LANA is expressed as an early gene upon primary infection of a HUVEC and BJAB cells and that there exists a reciprocal relationship between IE transactivator Rta/orf50 message and LANA message (Krishnan et al. 2004). Rta/orf50 mRNA peaks within minutes of infection, but then declines as LANA mRNA increases. The relative ratios depend on the host propensity to establish latent or lytic infection. In cells that support high-level KSHV lytic replication (HUVEC, HEK293), Rta/orf50 levels stay high as a significant proportion of

Table 20.2 Genome-wide profiling studies for KSHV viral gene transcription

Tissue	Platform	Citation
PEL		
BCBL-1	Reverse Northern Blot	(Zhong et al. 1996)
BCBL-1	70-mer array	(Wang et al. 2002)
BCBL-1	real-time QPCR	(Fakhari and Dittmer 2002)
BCBL-1	cDNA array	(Paulose-Murphy et al. 2001a)
BCBL-1/rapamycin	real-time QPCR	(Sin et al. 2007)
BCBL-1/cidofovir	cDNA array	(Lu et al. 2004)
BCBL-1/ganciclovir	real-time QPCR	(Staudt et al. 2004)
BC-1	Northern Blot	(Sarid et al. 1998)
BC-1	Real-time QPCR	(Whitby et al. 2007)
BC-3	cDNA array	(Jenner et al. 2001a)
JSC-1	real-time QPCR	Bagni et al. (submitted)
JSC-1	cDNA array	(Suscovich et al. 2004)
BCBL-1/K1	cDNA array	(Lee et al. 2002)
BCBL-1/Rta/orf50	cDNA array	(Nakamura et al. 2003)
BCBL-1/NotchIC	Real-time QPCR	(Chang et al. 2005)
Endothelial		
TIVE	Real-time QPCR	(An et al. 2006)
KS	Real-time QPCR	(Dittmer 2003)
Murine endothelial cells	Real-time QPCR	(Mutlu et al. 2007)
HMVEC-d	cDNA array	(Krishnan et al. 2004)
HMVEC-d	cDNA array	(Moses et al. 1999)
HUVEC	Real-time QPCR	(Yoo et al. 2005)

the culture enter complete lytic replication as characterized by subsequent E and L gene transcription. In cells that do not support KSHV lytic replication in the absence of external stimuli such as phorbol ester (BJAB, fibroblasts), Rta/orf50 levels rapidly decline as infected cells enter latency or loose the viral genome.

At times profiling studies also showed an increase of latent (LANA, vCYC, vFLIP) mRNA at late times after lytic reactivation (Jenner et al. 2001a), which would classify LANA as a gamma2 class mRNA (Fig. 20.1). A similar observation has recently been made in EBV (Yuan et al. 2006). We did not find evidence for significant latent mRNA induction at late times in BCBL-1 cells (Fakhari and Dittmer 2002); significant in as much, as it could not be explained by genome copy number amplification or changes in PEL culture composition. Unlike EBV-infected BL cell lines, in which the majority of cells reactivate from latency upon IgM cross-linking, in any PEL cell line never more than 50% (often less than 20%) of cells reactivate the virus in response to phorbol ester. This poses no problem for the study of lytic transcription, as lytic transcripts are virtually undetectable in latent cells and only cells that do reactivate contribute to the signal. However, it makes studies of latent gene transcription in response to stimuli difficult to interpret, since these are conducted in a background of uninduced cells.

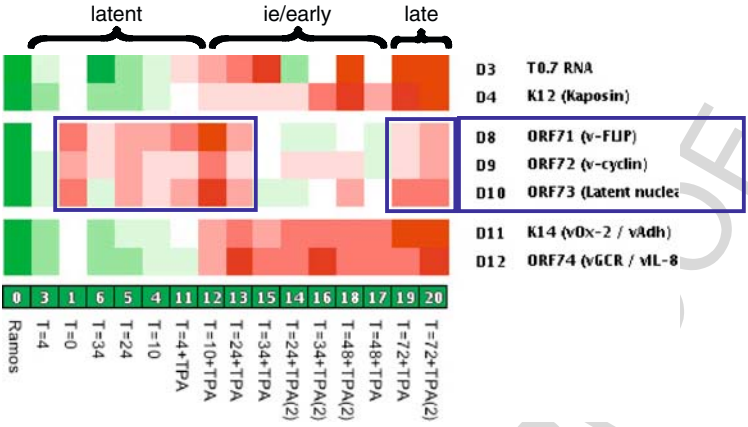

Fig. 20.1 Transcription profile of the KSHV latency locus (*See* Color Insert)
*Primary data from (Jenner et al. 2001a) were re-analyzed using ArrayMiner™

20.2 The KSHV Latency Locus

The KSHV latent genes LANA, vCYC and vFLIP (and miRs) all cluster together within one transcription unit (Fig. 20.2). At least one nascent transcript exists that traverses the entire locus (Pearce et al. 2005; Samols et al. 2005; Cai and Cullen 2006). It initiates at the constitutive LANA promoter start site at nt 127,880 and terminates at the *Kaposin*-distal poly-A site at nt 117,432. Transcription across this locus proceeds only in one direction: from right to left. To date no evidence for mRNAs originating at the opposite strand has been found. Furthermore, latent transcription was polymerase-II dependent.

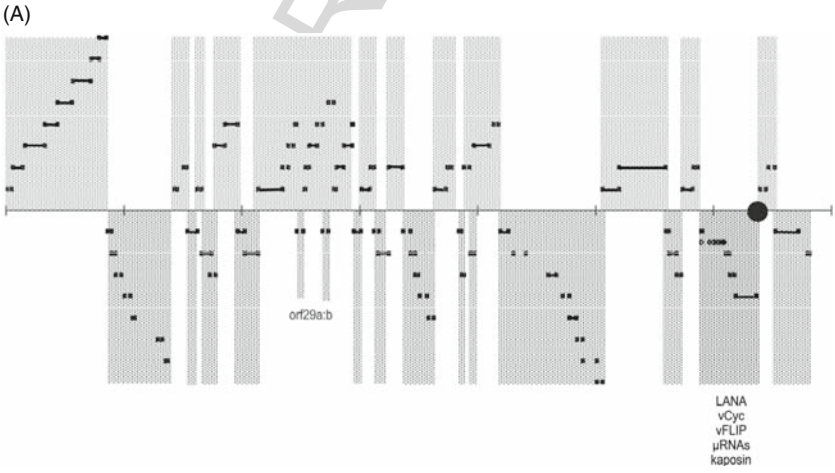

Fig. 20.2 (continued)

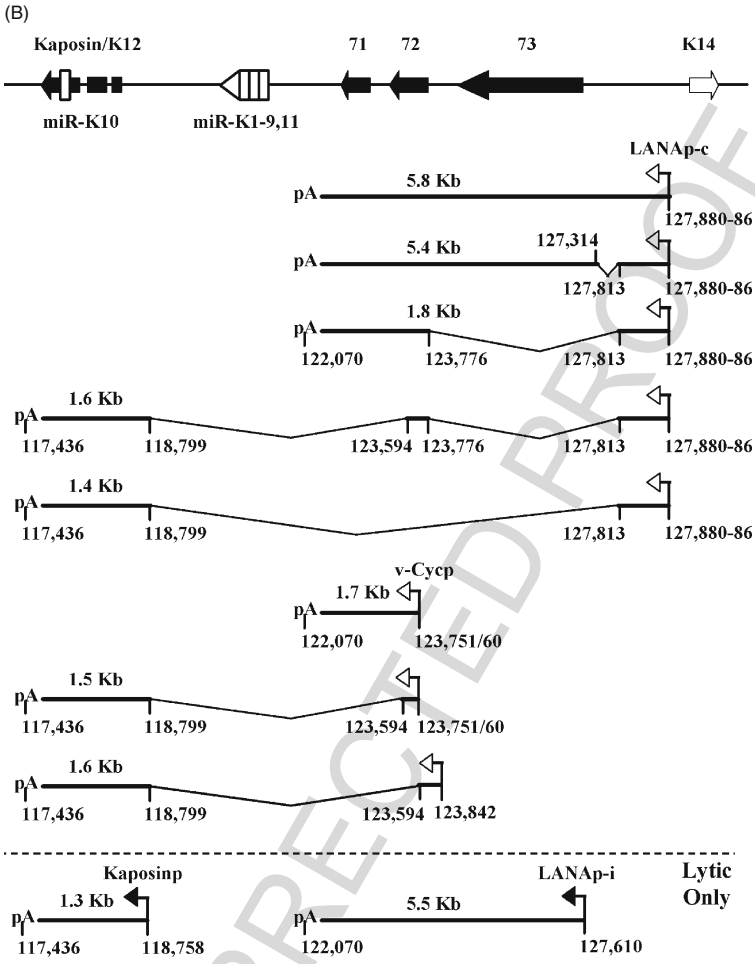

Fig. 20.2 The KSHV latency locus **(A) KSHV genome to scale.** Upper half, rightward orfs; lower half, leftward orfs, **(B) Transcript architecture of the major latency locus of KSHV (modified from Cai and Cullen (2006)).** The relative genomic position of KSHV open reading frames (ORFs) are shown along the top line as filled arrows to denote ORF directionality (*Kaposin*/*K12*; *ORF71*/*v-FLIP*; *ORF72*/*v-cyclin*; *ORF73*/*LANA*; *K14*/*v-Ox2*). The KSHV microRNAs (miR) are denoted as white boxes and are designated with the *K*-prefix (miR-K1-11). Transcripts expressed during latency in the absence of viral transactivators are denoted with white arrowheads; these originate at either the predominant LANAp-c or the weak v-Cycp. Transcripts dependent on the KSHV lytic-switch transactivator, Rta/ORF50, are denoted with black arrowheads and are shown below the dotted line for clarity. Lytic transcripts originate at either the LANAp-i or the *Kaposin*p. Genomic coordinates of transcript start sites and splice donors/acceptors are shown below the transcript diagram according to Russo et al. (1996a, b)

We are very confident in our assignment of the type I and type II latent genes, which show a dramatically different transcription profile that any of the other KSHV mRNAs regardless of experimental platform, PEL line or study (Fig. 20.1), and for which we have independent verification by in situ analysis (Dittmer et al. 1998; Dupin et al. 1999a; Katano et al. 1999, 2000; Parravicini et al. 2000; Rivas et al. 2001a). However, the PEL experimental system is far from perfect. At any given time 2–5% of PEL cells reactivate KSHV, such that highly expressed lytic mRNAs are detectable in uninduced cultures. The best example is early nut-1 RNA, which can be detected by Northern blot analysis of induced PEL (Zhong et al. 1996), since the few cells that express nut-1 make large amounts of RNA. *Kaposin* lytic transcripts, which originate from the gene proximal lytic promoter, likewise can be detected in latent PEL (Sadler et al. 1999), as can be those for K9/vIRF-1 (Chen et al. 2000). Yet, different latent mRNAs have been identified for K9/vIRF-1 and *Kaposin*, which map to a more distal constitutive start site. In addition, the latent genes respond to changes in the host cell. The spliced vCYC mRNA may be subjected to cell cycle regulation (Sarid et al. 1999a), or the cell cycle stage may influence the rate of spontaneous lytic reactivation (McAllister et al. 2005) thus changing the vCYC transcription pattern. In MCD, the vIL-6/K2 protein is expressed in a large percentage of cells, far more than any structural lytic protein (Staskus et al. 1999; Deng et al. 2002), suggesting that the vIL-6/K2 promoter can respond to MCD-specific transcription factors irrespective of the status of all other KSHV genes (Chatterjee et al. 2002).

Understanding KSHV transcription is a work in progress that requires careful molecular characterization of the viral regulatory elements. As we are starting to map the virus–host interactions at the transcriptional level, we gain fascinating insights into the pathogenesis of KSHV, which ultimately will lead to novel intervention targets.

20.2.1 LANA, vCYC, vFLIP

20.2.1.1 Latency-Associated Nuclear Antigen (LANA)

During latency, all KSHV-infected cells express the viral latency-associated nuclear antigen (LANA/ORF73) (Dittmer et al.; Dupin et al. 1999b). LANA is the predominant target of anti-KSHV antibodies in infected individuals. LANA is necessary and sufficient for latent viral episome persistence (Ballestas et al. 1999; Cotter and Robertson 1999; Hu et al.; Ye et al. 2004). Although LANA shows no homology at the DNA sequence level, its function and structural features are reminiscent of the Epstein–Barr virus EBNA-1 and EBNA-2 proteins. LANA contains a central region of acidic repeats, a leucine zipper, an N-terminal proline-rich domain and two nuclear localization sequences – one located at each termini of the polypeptide (diagrammed in Fig. 20.4). The acidic repeat regions and leucine zipper (EEDD, DE[E,Q]QQ

and LEEQEQEL between amino acids (aa) 338. . .840) and (LEEQEQEL at aa 840) are highly immunogenic and the target for the widely used commercial anti-LANA monoclonal antibody LN53 (Kellam et al. 1999).

The first function of LANA is to tether the viral episome to cellular chromatin and thus facilitate proper inheritance during cell division. The details of how LANA maintains the latent viral episome have been carefully studied. The C-terminus of LANA binds directly to two adjacent 16-bp motifs within the KSHV terminal repeats (TR), termed the LANAbinding sites 1 and 2 (LBS1 and LBS2). Both LBS share the conservation of 13 nucleotides, though nucleotides flanking this 13 nt. core are different between the two (Garber et al. 2001, 2002). LANA binds with higher affinity to LBS1 than to LBS2, suggesting the nucleotide sequence flanking the conserved 13 bp. LBS motif influences the binding affinity of LANA. This hypothesis has been directly investigated: Kaye and colleagues have found that nucleotides within and surrounding the LBS1 do affect LANA's-binding affinity for that site (Srinivasan et al. 2004).

LANA has been shown to bind cellular chromatin and mitotic chromosomes through its N-terminus (Shinohara et al. 2002). In this manner, LANA tethers the KSHV episome to cellular chromatin and chromosomes – thereby ensuring proper segregation of the viral genome during host cell division (Kedes et al. 1997b; Kellam et al. 1997b; Rainbow et al. 1997; Ballestas et al. 1999; Cotter and Robertson 1999; Szekely et al. 1999) (Kelley-Clarke et al. 2007a). Experimental abrogation of LANA expression through siRNA or genomic knockout leads to loss of KSHV episomes from latently infected cells, genetically demonstrating that LANA is necessary for maintenance of latency (Ye et al. 2004; Godfrey et al. 2005). Barbera et al. (2006) demonstrated that the N-terminus of LANA docks onto cellular chromosomes by directly binding to the folded regions of histones H2A and H2B to mediate nucleosome attachment (Barbera et al. 2006). Both histones H2A and H2B were necessary for LANA to bind nucleosomes. In contrast, Robertson and colleagues have found that LANA binds histone H1 (Barbera et al. 2006) as well as a host of other proteins involved in DNA structure remodeling (Verma et al. 2006a). Cellular replication and replication-licensing factors can also bind to LANA (Stedman et al. 2004; Lu et al. 2006; Verma et al. 2006b), suggesting that the KSHV episome– host chromatin interaction is not static but responds to viral latent replication (via the latent ori in the KSHV TRs) as well as host replication.

Second to facilitating episomal attachment, LANA can mediate transcriptional suppression. In the presence of LANA, LBS1 and LBS2 repress transcription of an artificial minimal promoter when placed upstream (Garber et al. 2001, 2002). The ability of LANA to carry out these functions is directly proportional to LANA's-binding affinity for these two sites. In the context of the viral genome, LANA can repress transcription of the K1 promoter via the LBS1 and LBS2 of the TRs (Verma et al.).

Other than by direct DNA binding, the transcriptional repressor function of LANA can also be mediated by cellular methyl transferases (Shamay et al.

2006). Currently there exists no evidence that this mechanism is used to modulate viral transcription. More likely, DNA-independent transcriptional silencing provides one avenue by which LANA reprograms the latently infected host cell. Ectopic expression of LANA leads to both up- and down-modulation over 147 cellular genes (Renne et al. 2001; An et al.) and this global reprogramming may provide one mechanism that mediates LANA's in vivo transforming function (Fakhari et al. 2006).

Third, LANA positively modulates the transcriptional activity of its own promoter (Jeonget al.; Renne et al. 2001; Jeong et al.). Although the LANA promoter (LANAp) is constitutively active in the absence of viral proteins, expression of LANA leads to auto-activation of its own promoter to maintain a positive-feedback loop. The C-terminus of LANA protein is required for auto-activation of the LANAp since deletions of amino acids (aa) 1002–1062 or 1113–1162 reduced LANAp auto-activation (Jeong et al.). This comprises the DNA-binding region of LANA (Kelley-Clarke et al. 2007b) and is also required for binding to KSHV TR sequences LBS1 and LBS2 (Garber et al. 2001, 2002). The central repeat domains of LANA that span aa 214–750 (including a proline-rich, a DE acidic repeat, and a Q-rich domain) are dispensable for auto-activation of the LANAp since expression of these deletion mutants still transactivated the LANAp to wild-type/full-length levels (Jeong et al.). This is not surprising since the central acidic repeat region of LANA is highly variable among KSHV isolates and is missing entirely from the rhesus rhadinovirus (RRV) LANA (Zhang et al. 2000; DeWire and Damania 2005).

LANA protein binds to nt. 127,903–127,923 of the core LANAp as demonstrated by EMSA (Jeong et al.). Sequence analysis of nt. 127,908–127,915 revealed the presence of an 8 bp motif that is centrally present with LBS1 within the KSHV terminal repeats (TRs). In contrast to each TR where two 16 nt. LBS exist in tandem, the LANA promoter contains only a single core LANA-binding site motif upstream of the latent 127,880 start site.

A detailed investigation has reported on LANA's transactivation of the cellular human telomerase reverse transcriptase (hTERT) promoter through interaction with cellular Sp1 protein (Verma et al.). Verma et al. showed that the C-terminus of LANA is necessary and sufficient to bind Sp1 protein. LANA was shown to bind to Sp1 via Sp1's glutamine-rich "B" domain, which is one of two (A and B) domains required for transcriptional activation (Courey et al. 1989; Verma et al.) but not DNA binding (Kardassis et al. 1999). This report implicates synergism between LANA and Sp1 to activate transcription on the cellular hTERT promoter. Of interest to this idea is the location of the core LANA-binding site within the core LANAp (127,908–920), which is adjacent to the Sp1-binding site (127,928–933) (Jeong et al. 2004). Based on these observations, it is likely that LANA and Sp1 synergize to activate LANAp transcription.

In addition to specific DNA binding, LANA can act as a promiscuous transcription co-factor on other promoters independent of its own DNA-binding recognition element through interaction with cellular proteins including:

Sp-1 (Verma et al.), RBP-jkappa (also known as CSL) (Lan et al. 2005a), p53 (Friborg et al. 1999), Rb (Radkov et al. 2000a, b), GSK-3beta (Fujimuro et al. 2003), CBP (Lim et al. 2000, 2001), ATF4/CREB2 (Lim et al. 2000, 2001), Ring3 (Platt et al. 1999) (Mattsson et al. 2002) (Viejo-Borbolla et al. 2005; Ottinger et al. 2006) and KSHV Rta/orf50 (Lan et al. 2005b). Chromatin-modifying factors SAP30, mSin3A and CIR (Krithivas et al. 2000, 2002), meCP2, DEK (Krithivas et al. 2000, 2002), Histone H1 (Cotter and Robertson 1999) and Histones H2A and H2B (Barbera et al. 2006) also can mediate these effects.

Finally, LANA binds to and inhibits Rb (Radkov et al. 2000b) as well p53 function in reporter assays (Friborg et al. 1999; Wong et al. 2004). Conversely, p53 can inhibit the LANA promoter (Jeong et al. 2001). This initially led to a model, in which LANA behaved very much like the small DNA tumor virus transforming proteins. In fact, because of its ability to decorate host chromosomes LANA can induce chromosome instability phenotypes that are akin to p53 inactivation (Pan et al. 2004; Si and Robertson 2006). However, the situation is more complex. At least one PEL cell line (BC-3) has lost Rb protein expression (Platt et al. 2002), which seems unnecessary if LANA efficiently counteracted all Rb functions, but it leaves open the possibility that LANA may interact with and inactivate other RB family members. LANA, of course, has multiple binding partners ($\geq$10) and functions (Si et al. 2006; Cai et al. 2006). These include Ku70, Ku80 and PARP-1, which can also be in complexes containing p53. Hence, it is easy to rationalize how some LANA can be found in complex with p53. Despite being in complex with LANA, p53 is fully functional in PEL (Petre et al. 2007) and can be activated by doxorubicin. Moreover, the LANA-p53 complex can be destroyed by the mdm-2/p53 interaction inhibitor nutlin (Petre et al. 2007; Sarek et al. 2007), which leads to p53-dependent apoptosis in PEL.

20.2.1.2 v-CYC/orf72

v-cyclin (orf72) represents another candidate KSHV oncogene because of its homology to the human cyclin-D/Prad oncogene. In general, cyclin-D proteins (D_1, D_2, D_3) associate with specific cyclin-dependent kinases (CDKs) and these complexes phosphorylate Rb family members (reviewed in Sherr (1996)). This in turn liberates E2F/DP-1 transactivation functions that are necessary and sufficient for S-phase entry. Importantly, the human cyclin-D_1 gene is amplified in parathyroid tumors, a subset of prostate and breast cancers as well as human mantle cell lymphomas. It can complement *ras* in transforming low passage rodent cells in culture (Hinds et al. 1994; Lovec et al. 1994) as well as *c-myc* in transgenic mice (Bodrug et al. 1994). An oncogenic cyclin-D homolog is also present in other gamma herpesviruses (reviewed in Neipel et al. (1997)). Ectopic expression of the murine herpesvirus 68 (MHV68) cyclin in T cells causes T-cell lymphomas in transgenic mice (van Dyk et al. 1999).

The mechanism of transformation by KSHV v-cyclin is most likely novel and unique, since it phosphorylates pRb but, unexpectedly, also histone H1, $p27^{KIP1}$ and bcl-2 (Chang et al. 1996; GoddenKent et al. 1997; Li et al. 1997; Ojala et al. 2000; Laman et al. 2001). Unlike human cyclin-D, v-cyclin/cdk6-mediated phosphorylation of Rb is resistant to inhibition by the cyclin-dependent-kinase-inhibitors (CDKIs) $p16^{INK4}$, $p21^{CIP1}$ and $p27^{KIP1}$ (Swanton et al. 1997). Moreover, v-cyclin/cdk6 induces the degradation of $p27^{KIP1}$ (Ellis et al. 1999; Mann et al. 1999). Yet, the results of these transient expression studies remain controversial: v-cyclin can overcome a $p16^{INK4}$ G_1 arrest (Swanton et al. 1997), but its activation of the E2F-responsive cyclin A promoter is inhibited by $p16^{INK4}$(Duro et al. 1999). Depending on the cell line used, v-cyclin binds exclusively to cdk6 (GoddenKent et al. 1997), to cdk4 and cdk6 (Li et al. 1997) or to cdk4, cdk6 and cdk2 (Mann et al. 1999).

Despite significant overall sequence identity, key residues required for cyclin D1 nuclear export and degradation are lacking in the K-cyclin C-terminus . As a result, K-cyclin possesses a longer half life than cyclin D1 and displays more pronounced nuclear accumulation. In the case of human cyclin D, a mutant allele (K112E or K114E) has been generated, which is incapable of activating CDKs. Mutation of the homologous K-cyclin residue (K106 to E) significantly (~50%) reduced CDK6 interaction as well as RB phosphorylation. Recent evidence (Upton and Speck 2006) suggests that the homologous, cdk-binding deficient mutant in the murid herpesvirus 68 (MHV-68) viral cyclin D homolog was able to replicate in culture, but was attenuated for replication in vivo.

V-cyclin over-expression induces transient proliferation (Swanton et al. 1997), as well as apoptosis (Ojala et al. 1999; Hardwick 2000; Ojala et al. 2000). To date, no stable cell lines that express v-cyclin have been reported, suggesting that high-level expression of v-cyclin is not compatible with continued cell growth. However, loss of p53 uncovered the transforming potential of vCYC in vivo. While KSHV vCYC single transgenic mice did not develop tumors, lymphomas developed rapidly in a p53null background (Verschuren et al. 2002, 2004). An analogous phenotype has been observed in at least one transgenic model for human cyclin D1, where either deletion or targeted overexpression of wild-type cyclin D1 in photoreceptor cells was associated with apoptosis (Fantl et al. 1995; Ma et al. 1998; Skapek et al. 2001). Presumably, loss of p53 counteracted the pro-apoptotic signals that were associated with forced KSHV vCYC expression. In contrast to KSHV, 60% of transgenic mice expressing the MHV-68 cyclin D homolog in T cell developed lymphoma within 12 months (van Dyk et al. 1999). This suggests that cell lineage and the differentiation state of the host cell and cyclin needed to be in the right balance. This data suggest a model that requires multiple events initiated by the concerted action of all KSHV latent proteins for KSHV-dependent lymphomagenesis.

20.2.1.3 vFLIP/orf71

v-FLIP/orf71 is transcribed from the LANA promoter and translated from an internal ribosome entry site located within the vCYC coding region (Grundhoff

and Ganem 2001) (Low et al. 2001). The vFLIP protein has sequence homology to equine herpesvirus-2 E8 and herpesvirus Saimiri (HVS) orf71 (Hu et al. 1997). It inhibits CD95/FAS-induced apoptosis in vitro by blocking caspase-3, -8 and -9 (Djerbi et al. 1999). Both CD95/Fas-L and TRAIL/TNF-alpha induce apoptosis through a similar mechanism (Muzio et al. 1996; Medema et al. 1997). Clustering of the receptor upon binding of the ligand recruits an adapter molecule (FADD and TRADD, respectively) with a binding domain (DD) for the receptor and a conserved "death-effector-domain" (DED) that binds and triggers the activation of caspase-8. The death signal is then transduced through a number of cellular caspases resulting in the commencement of cellular apoptosis (for review see Hu et al. 1997). A possible mechanism for viral FLIPs postulates competition with the adapter molecule for binding to caspase 8 via its DED domain.

A more recent line of inquiry found vFLIP to be involved in NFkappaB signaling. Here, vFLIP uses its TRAF-binding domain to activate NFkappaB signaling (Guasparri et al. 2006). vFLIP activated IkkappaB-kinase (An et al. 2003; Field et al. 2003) and thereby increases NF-kappaB activity, which is anti-apoptotic in PEL cell. In addition, vFLIP induced MHC-I expression through NF-kappaB in KSHV-infected lymphatic endothelial cells (Lagos et al. 2007), which underscores the physiological importance of the vFLIP-NF-kappaB interaction. Moreover, vFLIP transgenic mice develop lymphoma (Chugh et al. 2005). Eliminating either vFLIP or NF-kappaB activity from PEL induces apoptosis (Keller et al. 2000; Guasparri et al. 2004; Godfrey et al. 2005), demonstrating that this pathway is essential for lymphomagenesis.

20.2.2 KSHV miRNAs

Micro RNAs (miRNAs) are a novel class of mammalian genes. They regulate the transcription and translation of many target proteins and have been implicated in normal development as well as carcinogenesis. Viruses also encode miRNAs. In KSHV, the miRNAs are conserved among different isolates (Marshall et al. 2007) and grouped together in the viral latency region (nucleotide 119305–121911) (Cai et al. 2005; Pfeffer et al. 2005; Samols et al. 2005). This organization is similar to mammalian miRNA gene organization where clustering has been observed for 50–70% of miRNA genes (Altuvia et al. 2005). The maturation of miRNAs is the subject of active research (reviewed in (Cullen 2004)). First, a primary miRNA, or pri-miRNA, is transcribed by RNA polymerase II. It is capped and polyadenylated in the nucleus (Cai et al. 2004). The pri-miRNA can be of any length and contain any number of clustered miRNAs. The KSHV miRNAs are derived from a common precursor molecule (Pearce et al. 2005; Cai and Cullen 2006), comparable to the stable HSV-1 lat intron (Cui et al. 2006). They share a common leftward-orientation and are regulated by multiple splicing events, multiple

termination sites and multiple transcription initiation sites (at nt 127,880, 123,848, 118,758) (Dittmer et al. 1998; Sadler et al. 1999; Sarid et al. 1999a; Talbot et al. 1999; Li et al. 2002; Marshall et al. 2007). Promoter–reporter constructs encompassing these three latent start sites all exhibited activity after transient transfection, but by comparison the constitutive LANA promoter at nt 127,880 exhibited 20-fold higher basal activity then the vCYC promoter (O'Hara and Dittmer, unpublished observation). This pattern of promoter activity is consistent with a model whereby the common LANA promoter regulates all KSHV latent RNA species, giving rise to mRNAs as well as all miRNAs in KSHV-associated cancers.

The pri-miRNA serves as substrate for the Drosha nuclease complex (Zeng et al. 2005). Through Drosha the precursor miRNAs, or pre-miRNAs, are generated, each serving as the precursor of one or two mature miRNAs. The pre-miRNAs reside in the nucleus and are ~70 nucleotides in length. The stability of the pre-miRNAs can vary (Schmittgen et al. 2004; Pfeffer et al. 2005). In KSHV, the pre-miRNAs are stable as they can be detected by Northern hybridization. Overall their levels correlate with the level of the mature KSHV miRNAs (Cai et al. 2005; Pfeffer et al. 2005); Samols et al. 2005) (see reference (Gottwein et al. 2006) for an exception) and can be detected in all PEL cell lines as well as in primary KS biopsies (O'Hara and Dittmer, submitted). The pre-miRNAs are subsequently exported out of the nucleus with the help of Exportin 5 and serve as a substrate for Dicer in the cytoplasm. Mature miRNA levels can be regulated by modulating exportin-5 expression (Yi et al. 2005). In the cytoplasm, Dicer processes the pre-miRNA into the mature miRNA and complementary strand, each comprising ~22 nt in length. For some miRNAs, both the sense and the anti-sense pre-miRNA strands serve as template for mature miRNAs. For KSHV, this has been demonstrated for miR-K4, miR-K6 and miR-K9. The mature miRNAs are then incorporated into the RISC complex, which carries out the enzymatic function.

The elucidation of miRNA targets and the function that the KSHV miRNAs play in the viral life cycle is the subject of active research and covered in detail in Chapter 25.

20.2.3 Kaposin/K12

Kaposin is located immediately downstream of LANA, vCYC and vFLIP and in addition to the common promoter can be regulated by a promoter located between LANA and cyclin (Li et al. 2002) and during lytic reactivation yet another, orf-proximal promoter (Sadler et al. 1999). Like LANA, *Kaposin* too is expressed in every tumor cell (Staskus et al. 1997). In fact, *Kaposin* mRNA is the most abundant mRNA in latently infected PEL. It gives rise to an interesting group of alternatively translated proteins (Sadler et al. 1999), at least some of which can transform NIH3T3 cells in culture (Muralidhar et al. 1998). *Kaposin*

interacts with cytohesin-1 (Kliche et al. 2001). In addition, it has been shown to stabilize cellular cytokine mRNAs through the p38 mitogen-activated protein kinase (MAPK)/MK2 kinase pathway (McCormick and Ganem 2005; McCormick and Ganem 2006). Interestingly, and perhaps because of its high expression and protein repeats, *Kaposin* provides target peptides for the human CD8 cytotoxic T-cell response (Brander et al. 2001); Micheletti et al. 2002).

20.3 LANA-2/vIRF-3 and K9/vIRF-1

Profiling of KSHV mRNAs in PEL revealed one new lated orf that was not included within the LANA latency locus (Fakhari and Dittmer 2002). It belonged to a KSHV vIRF homolog, also called LANA-2/v-IRF-3, which is not expressed in KS, but is expressed in 100% of PEL and MCD in a pattern similar to LANA (Lubyova and Pitha 2000; Rivas et al. 2001a). LANA-2/vIRF-3 counteracts cellular IRFs, but also p53 function (Rivas et al. 2001a). LANA-2/vIRF-3 is a member of several KSHV IRF homologs. Their function in immune evasion is described in detail in elsewhere (see Chapter by J. Jung). The viral IRFs, just like the viral latent genes are clustered and oriented as repeats of leftward orfs (see Table 20.3). There transcription is complex and not fully understood. LANA2/vIrf-3 is only expressed in B lineage cells, but here in every cell, whereas for K9/vIRF-1 has both latent and lytic transcriptional start sites (Chen et al. 2000) have been described in PEL. More important, by cluster analysis, K9/vIRF-1 clustered with the other latency genes in endothelial-cell lineage KS tumors (Dittmer 2003), as if at least one vIRF has to be expressed during viral latency.

Table 20.3 KSHV encodes multiple viral interferon regulatory genes as clustered leftward orfs

Name	Location based on (Russo et al. 1996b)	Transcriptional class
orf57/Mta/Sm (right)		IE
	polyA-termination: 83637	
K9/vIRF-1	83860–85209	E
	Upstream start (-84)	LATENT
	polyA-termination: 86006	
K10 s	88085–86074	LATENT
K10:	88164–86074	LYTIC
K10-K10.1/vIR-4	88910–86076 (intron: 88443–88343)	LYTIC
K10-K10.1	88910–86076 (intron: 88443–88343 intron: 89034–88799)	LATENT
K10.5-K10.6 (or 10.7)/ LANA-2/ vIRF-3	91393–89599 (intron: 90938–90847)	LATENT
K11:	91964–93367	LYTIC
K11-K11.2:	94123–91964 (intron: undefined)	LYTIC
K11.2/ vIRF-2	94123–93623	LYTIC

20.4 Architecture of the KSHV Latency Locus Promoter (LANAp)

LANA transcription is regulated by the LANA promoter (LANAp), depicted in Fig. 20.3 (Dittmer et al.; Sarid et al.; Talbot et al.). In its initial characterizations, the LANAp was found to direct transcription of poly-cistronic mRNAs encoding either LANA/ORF73, v-cyclin/ORF72, and v-FLIP/ORF71 or only v-cyclin/ORF72 and v-FLIP/ORF71 through alternative splicing out of LANA/ORF73 (Dittmer et al.; Sarid et al.) (diagrammed in Fig. 20.2, *top three transcripts*). Transcriptional profiling of viral gene expression showed that during viral latency in PEL and in primary KS biopsies, LANA, v-cyclin, v-FLIP and *Kaposin* were constitutively expressed (Jenner et al.; Paulose-Murphy et al.; Fakhari and Dittmer; Dittmer). Therefore, under conditions where other KSHV promoters were silenced, the LANA promoter remained constitutively active. GpC islands within the LANAp are constitutively unmethylated in both PEL and KS (Chen et al. 2001) and are associated with an "open" chromatin environment. This is in contrast to, for instance, the promoter for the KSHV lytic-switch protein Rta/ORF50. Treatment with sodium butyrate, an inhibitor of histone deacetylases (HDACs), did not change the acetylation status of histones H3 and H4 on the LANAp since the promoter was already de-repressed, contrasting the response observed on the Rta/ORF50 promoter (Lu et al. 2003).

Left of the LANA/v-cyclin/v-FLIP locus is another latently expressed gene, *Kaposin*/*K12*. While *Kaposin*/*K12* has its own promoter that is highly responsive to the lytic-switch protein, Rta/ORF50. Recent work has discovered transcripts containing *Kaposin*/K12 message originating from a weak promoter located in front of v-cyclin/ORF72 (Pearce et al.; Cai and Cullen) and also originating from the latent LANAp (Cai and Cullen). Importantly, the newly discovered KSHV microRNAs (miRNAs) are located within the intergenic region between the v-FLIP/ORF71 and the *Kaposin*/K12 open reading frames (Cai et al.; Pfeffer et al.; Samols et al.). Therefore, transcripts that originate from the latent

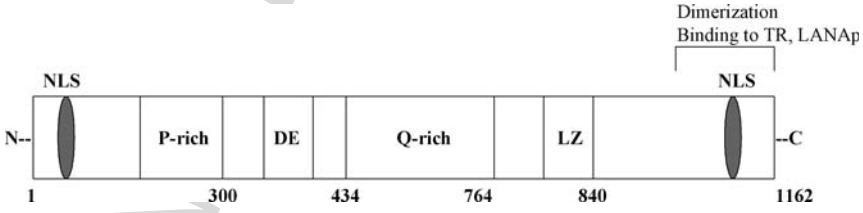

Fig. 20.3 Domain structure of LANA **Diagram of the KSHV Latency-Associated Nuclear Antigen, LANA (with permission from M. Staudt, 2006).** The gene product of *ORF73* is the KSHV latency-associated nuclear antigen (LANA). LANA is an 1162 amino acid (aa) protein that contains a nuclear localization sequence (NLS) at both the N- and C-termini, a proline-rich domain (P-rich), an acidic repeat domain of aspartic and glutamic acid (DE), a glutamine-rich domain (Q) and a leucine zipper domain (LZ). The C-terminal 231 aa facilitates binding to genomic terminal repeat (TR) DNA and to LANA promoter DNA

LANAp and encode the *Kaposin*/K12 ORF also encode the miRNAs, and as such can explain the constitutive expression of the KSHV miRNAs during latency (Cai and Cullen 2006) (see Fig. 20.2 for diagram of transcripts). These novel reports demonstrate that despite its name, the LANAp can also direct expression of every viral gene expressed during latency. An exception to this is the latent PEL-specific LANA-2/vIRF3, which is located in a distant genomic location outside of the LANA latency locus (Rivas et al. 2001a, b). Spatial clustering and 5′-co-terminal regulation underscores the importance of this region and sets the latency-associated locus and the LANAp apart from all other viral transcription regions.

The LANAp is constitutively active in the absence of viral proteins in all cell lines tested, including KSHV-positive and -negative B cells, HEK293 epithelial cells and SLK endothelial cells (Jeong et al.; Jeong et al.). Moreover, a 1,861 bp DNA fragment originating at the LANA AUG at position 127,300 and extending to position 129,161 (–1299 bp relative to the latent transcription start site) was able to direct B cell-specific reporter gene expression in transgenic mice (Jeong et al.). This demonstrated that host cell transcription factors in the absence of any viral transactivators suffice to direct LANAp activity and, by inference, LANA, v-cyclin, v-FLIP, *Kaposin*/K12 and miRNA transcription during viral latency.

Previous reports on LANAp deletion analyses mapped the core promoter region from +10 to –88 (nt. 127,870–127,968) relative to the latent transcription start site at 127,880 (Dittmer et al.; Jeong et al.). While the core promoter mediates minimal LANAp activity, the presence of additional promoter sequence both up- and downstream of the core promoter significantly contributed to LANAp activity (Jeong et al.). The presence of distal sequences from –88 up to –279 (nt. 127,968–128,159) as well as sequences within the 5′UTR from +10 down to +271 (nt. 127,870–127,609) enhanced reporter activity more than 10-fold relative to the minimal core promoter (Jeong et al.).

Although the LANAp exhibits constitutive activity in the absence of viral proteins, expression of LANA protein leads to an increase in promoter activity (Jeong et al.; Renne et al. 2001). Presumably central to this function, LANA has been shown to directly bind within its own promoter to a region that contains a small 8 bp consensus motif that is centrally located within the larger 16 bp. LANA-binding site 1 (LBS1) of the KSHV terminal repeats (TRs) (Garber et al. 2001, 2002). Enhancement of LANAp activity by LANA protein establishes a self-stabilizing feedback loop to maintain KSHV latency (Renne et al. 2001; Chiou et al.; Wong et al.).

The mammalian core promoter is generally defined as the minimal stretch of contiguous DNA sequence that is sufficient to direct accurate initiation of transcription by the RNA polymerase II machinery. They typically encompasses DNA sequences between approximately +50 and –40 relative to a transcriptional start site (Weis and Reinberg; Javahery et al. 1994; Smale 1997; Smale 2001). Several sequence motifs are commonly found in mammalian core promoters: these include the TATA box, initiator (Inr), the TFIIB

recognition element (BRE) and the downstream core promoter element (DPE). Each core promoter element can be found in some but not all core promoters, and it is a misconception that all promoters must contain each element. The core LANAp has been defined as +10 to –88 (Jeong et al.; Jeong et al.) and it contains the expected mammalian core promoter elements as well as an essential Sp1 site. These elements are independently required as shown by site-directed mutagenesis (Staudt and Dittmer 2006).

Other than during asymptomatic latency in B cells (Mesri et al.; Dittmer et al.) or in KSHV-associated tumors (Dittmer et al.; Dupin et al. 1999b), LANA protein and LANAp originating mRNAs have also been detected immediately after de novo infection of permissive endothelial cells and non-permissive fibroblasts (Krishnan et al. 2004; Yoo et al. 2005). In these experiments, KSHV rapidly establishes latency but can be reactivated by TPA. LANA and Rta/ORF50 mRNAs were described as immediate early mRNAs upon de novo infection. This prompted the discovery of a novel lytic-phase LANA promoter (Lan et al. 2005b; Matsumura et al. 2005, Staudt and Dittmer 2006).

The LANAp-c (127,880) is constitutively active during all forms of latency; its activity is enhanced by LANA (Jeong et al.) and independent of Rta/ORF50. A second, novel, downstream start site is only active in the presence of Rta/ORF50. Nucleotides 127,607–127,675 are sufficient and required for Rta/ORF50-responsiveness and encompass the core elements of the LANAp-i.

Interestingly, the LANAp-i was significantly more responsive to Rta/ORF50 in isolation than when linked to the LANAp-c as in the LANA-FL reporter. This effect could be a result of transcript elongation ensuing from the LANAp-c through the *LANA/ORF73* 5′ -UTR (containing the LANAp-i), which might prevent initiation events on LANAp-i *cis* regions. Such a mechanism was previously reported for the *GAL10* and *GAL7* promoters of *Saccharomyces cerevisiae* and for transcription through tandem HIV-1 promoters (Greger et al. 1998; Greger and Proudfoot 1998). In support of this notion, deletions of core LANAp-c regions that decreased basal promoter activity were associated with increased Rta/ORF50-responsiveness.

In the opposite direction of LANA and the latent transcripts is K14 and the vGCPR (see Fig. 20.4). The vGPCR promoter is absolutely dependent on Rta/

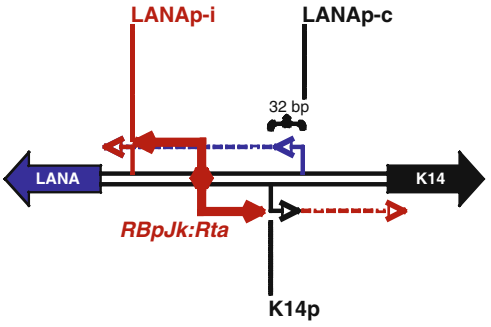

Fig. 20.4 The LANA promoter

orf50 (Liang and Ganem 2004; Liang et al. 2002). Mutation of a shared consensus RBP-jκ site at 127,736–127,740 reduced the ability of Rta/ORF50 to transactivate both the LANAp-i and the K14 promoters (Staudt and Dittmer 2006). These data suggest a mechanism whereby LANA transcripts derived from the LANAp-i can be transcribed during lytic reactivation without polymerase interference by K14/vGPCR transcripts that are simultaneously being transcribed on the complementary strand in the opposite orientation as a result of bi-directional transactivation from the KSHV lytic-switch protein, Rta/ORF50.

During de novo infection, Rta/ORF50 is present within KSHV virions (Bechtel et al.; Lan et al.) and as such is delivered into the host cell upon infection in the absence of LANA protein expression. Therefore, based on the data reported herein we speculate that Rta/ORF50 protein could initially transactivate the LANAp-i and K14/vGPCR promoters through direct DNA binding or via the shared consensus RBP-jκ site during de novo infection. As LANA protein expression ensues and LANA accumulates within the cell, expression of Rta/ORF50 protein is silenced as a result of LANA repression of the Rta/ORF50 promoter and LANA's inhibition of Rta/ORF50's transactivation function (Lan et al.). As a result, the LANAp-i and K14/vGPCR promoter activity would cease and LANA-coding mRNA could be transcribed from the latent LANAp-c, which is auto-regulated by LANA protein. This sequence of events can establish a positive-feedback loop that is sufficient to initiate and maintain viral latency within a permissive cellular environment.

Acknowledgment Work in the authors group is supported by funding from the Leukemia and Lymphoma Society and NIH (CA109232). Thanks to Drs. Blossom A. Damania and Michelle R. Staudt for illuminating discussions.

References

Ablashi DV, Chatlynne LG, Whitman JE, Jr., Cesarman E (2002) Spectrum of Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8, diseases. Clin Microbiol Rev 15:439–464

Altuvia Y et al. (2005) Clustering and conservation patterns of human microRNAs. Nucleic Acids Res 33:2697–2706

An FQ et al. (2006) Long-Term-Infected Telomerase-Immortalized Endothelial Cells: a Model for Kaposi's Sarcoma-Associated Herpesvirus Latency In Vitro and In Vivo. J Virol 80:4833–4846

An FQ, Compitello N, Horwitz E, Sramkoski M, Knudsen ES, Renne R (2005) The latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus modulates cellular gene expression and protects lymphoid cells from p16 INK4A-induced cell cycle arrest. J Biol Chem 280:3862–3874

An J, Sun Y, Sun R, Rettig MB (2003) Kaposi's sarcoma-associated herpesvirus encoded vFLIP induces cellular IL-6 expression: the role of the NF-kappaB and JNK/AP1 pathways. Oncogene 22:3371–3385

Antman K, Chang Y (2000) Kaposi's sarcoma. N Engl J Med 342:1027–1038

Ballestas ME, Chatis PA, Kaye KM (1999) Efficient persistence of extrachromosomal KSHV DNA mediated by latency-associated nuclear antigen. Science 284:641–644

Barbera AJ et al. (2006) The nucleosomal surface as a docking station for Kaposi's sarcoma herpesvirus LANA. Science 311:856–861

Bechtel JT, Winant RC, Ganem D (2005) Host and viral proteins in the virion of Kaposi's sarcoma-associated herpesvirus. J Virol 79:4952–4964

Bodrug SE, Warner BJ, Bath ML, Lindeman GJ, Harris AW, Adams JM (1994) Cyclin D1 transgene impedes lymphocyte maturation and collaborates in lymphomagenesis with the myc gene. Embo J 13:2124–2130

Brander C et al. (2001) Definition of an optimal cytotoxic T lymphocyte epitope in the latently expressed Kaposi's sarcoma-associated herpesvirus kaposin protein. J Infect Dis 184:119–126

Cai X, Cullen BR (2006) Transcriptional origin of Kaposi's sarcoma-associated herpesvirus microRNAs. J Virol 80:2234–2242

Cai X, Hagedorn CH, Cullen BR (2004) Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. Rna 10:1957–1966

Cai QL, Knight JS, Verma SC, Zald P, Robertson ES (2006) EC5S ubiquitin complex is recruited by KSHV latent antigen LANA for degradation of the VHL and p53 tumor suppressors. PLoS Pathog 2:e116

Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR (2005) Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. Proc Natl Acad Sci U S A 102:5570–5575

Chang H, Dittmer DP, Shin YC, Hong Y, Jung JU (2005) Role of Notch signal transduction in Kaposi's sarcoma-associated herpesvirus gene expression. J Virol 79:14371–14382

Chang Y et al. (1996) Cyclin encoded by KS herpesvirus [letter]. Nature 382:410

Chatterjee M, Osborne J, Bestetti G, Chang Y, Moore PS (2002) Viral IL-6-induced cell proliferation and immune evasion of interferon activity. Science 298:1432–1435

Chen J et al. (2001) Activation of latent Kaposi's sarcoma-associated herpesvirus by demethylation of the promoter of the lytic transactivator. Proc Natl Acad Sci U S A 98:4119–4124

Chen J, Ueda K, Sakakibara S, Okuno T, Yamanishi K (2000) Transcriptional Regulation of the Kaposi's Sarcoma-Associated Herpesvirus Viral Interferon Regulatory Factor Gene. J Virol 74:8623–8634

Chiou CJ et al. (2002) Patterns of gene expression and a transactivation function exhibited by the vGCR (ORF74) chemokine receptor protein of Kaposi's sarcoma-associated herpesvirus. J Virol 76:3421–3439

Chugh P et al. (2005) Constitutive NF-{kappa}B activation, normal Fas-induced apoptosis, and increased incidence of lymphoma in human herpes virus 8 K13 transgenic mice. Proc Natl Acad Sci U S A 102:12885–12890

Cotter MA, 2nd, Robertson ES (1999) The latency-associated nuclear antigen tethers the Kaposi's sarcoma-associated herpesvirus genome to host chromosomes in body cavity-based lymphoma cells. Virology 264:254–264

Courey AJ, Holtzman DA, Jackson SP, Tjian R (1989) Synergistic activation by the glutamine-rich domains of human transcription factor Sp1. Cell 59:827–836

Cui C et al. (2006) Prediction and identification of herpes simplex virus 1-encoded microRNAs. J Virol 80:5499–5508

Cullen BR (2004) Transcription and processing of human microRNA precursors. Mol Cell 16:861–865

Deng H, Song MJ, Chu JT, Sun R (2002) Transcriptional regulation of the interleukin-6 gene of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus). J Virol 76:8252–8264

DeWire SM, Damania B (2005) The Latency-Associated Nuclear Antigen of Rhesus Monkey Rhadinovirus Inhibits Viral Replication through Repression of Orf50/Rta Transcriptional Activation. J. Virol. 79:3127–3138

Dittmer DP (2003) Transcription profile of Kaposi's sarcoma-associated herpesvirus in primary Kaposi's sarcoma lesions as determined by real-time PCR arrays. Cancer Res 63:2010–2015

Dittmer D et al. (1999) Experimental transmission of Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) to SCID-hu Thy/Liv mice. J Exp Med 190:1857–1868

Dittmer D, Lagunoff M, Renne R, Staskus K, Haase A, Ganem D (1998) A cluster of latently expressed genes in Kaposi's sarcoma-associated herpesvirus. J Virol 72:8309–8315

Djerbi M, Screpanti V, Catrina AI, Bogen B, Biberfeld P, Grandien A (1999) The inhibitor of death receptor signaling, FLICE-inhibitory protein defines a new class of tumor progression factors [see comments]. J Exp Med 190:1025–1032

Dourmishev LA, Dourmishev AL, Palmeri D, Schwartz RA, Lukac DM (2003) Molecular genetics of Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) epidemiology and pathogenesis. Microbiol Mol Biol Rev 67:175–212, table of contents

Dupin N et al. (1999a) Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. Proceedings of the National Academy of Science USA 96:4546–4551

Dupin N et al. (1999b) Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. Proc Natl Acad Sci U S A 96:4546–4551

Duro D, Schulze A, Vogt B, Bartek J, Mittnacht S, Jansen-Durr P (1999) Activation of cyclin A gene expression by the cyclin encoded by human herpesvirus-8. J Gen Virol 80:549–555

Ellis M et al. (1999) Degradation of p27(Kip) cdk inhibitor triggered by Kaposi's sarcoma virus cyclin-cdk6 complex. Embo J 18:644–653

Fakhari FD, Dittmer DP (2002) Charting Latency Transcripts in Kaposi's Sarcoma-Associated Herpesvirus by Whole-Genome Real-Time Quantitative PCR. J Virol 76:6213–6223

Fakhari FD, Jeong JH, Kanan Y, Dittmer DP (2006) The latency-associated nuclear antigen of Kaposi sarcoma-associated herpesvirus induces B cell hyperplasia and lymphoma. J Clin Invest 116:735–742

Fantl V, Stamp G, Andrews A, Rosewell I, Dickson C (1995) Mice lacking cyclin D1 are small and show defects in eye and mammary gland development. Genes Dev 9:2364–2372

Field N et al. (2003) KSHV vFLIP binds to IKK-gamma to activate IKK. J Cell Sci 116:3721–3728

Friborg J, Jr., Kong W, Hottiger MO, Nabel GJ (1999) p53 inhibition by the LANA protein of KSHV protects against cell death. Nature 402:889–894

Fujimuro M et al. (2003) A novel viral mechanism for dysregulation of beta-catenin in Kaposi's sarcoma-associated herpesvirus latency. Nat Med 9:300–306

Garber AC, Hu J, Renne R (2002) Latency-associated nuclear antigen (LANA) cooperatively binds to two sites within the terminal repeat, and both sites contribute to the ability of LANA to suppress transcription and to facilitate DNA replication. J Biol Chem 277:27401–27411

Garber AC, Shu MA, Hu J, Renne R (2001) DNA binding and modulation of gene expression by the latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus. J Virol 75:7882–7892

GoddenKent D et al. (1997) The cyclin encoded by Kaposi's sarcoma-associated herpesvirus stimulates cdk6 to phosphorylate the retinoblastoma protein and histone H1. J Virol 71:4193–4198

Godfrey A, Anderson J, Papanastasiou A, Takeuchi Y, Boshoff C (2005) Inhibiting primary effusion lymphoma by lentiviral vectors encoding short hairpin RNA. Blood 105:2510–2518

Gottwein E, Cai X, Cullen BR (2006) A novel assay for viral microRNA function identifies a single nucleotide polymorphism that affects Drosha processing. J Virol 80:5321–5326

Greger IH, Demarchi F, Giacca M, Proudfoot NJ (1998) Transcriptional interference perturbs the binding of Sp1 to the HIV-1 promoter. Nucleic Acids Res 26:1294–1301

Greger IH, Proudfoot NJ (1998) Poly(A) signals control both transcriptional termination and initiation between the tandem GAL10 and GAL7 genes of Saccharomyces cerevisiae. Embo J 17:4771–4779

Grundhoff A, Ganem D (2001) Mechanisms governing expression of the v-FLIP gene of Kaposi's sarcoma-associated herpesvirus. J Virol 75:1857–1863.

Guasparri I, Keller SA, Cesarman E (2004) KSHV vFLIP is essential for the survival of infected lymphoma cells. J Exp Med 199:993–1003

Guasparri I, Wu H, Cesarman E (2006) The KSHV oncoprotein vFLIP contains a TRAF-interacting motif and requires TRAF2 and TRAF3 for signalling. EMBO Rep 7:114–119

Hardwick JM (2000) Cyclin' on the viral path to destruction. Nat Cell Biol 2:E203-E204

Hinds PW, Dowdy SF, Eaton EN, Arnold A, Weinberg RA (1994) Function of a human cyclin gene as an oncogene. Proc Natl Acad Sci U S A 91:709–713

Hu J, Garber AC, Renne R (2002) The latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus supports latent DNA replication in dividing cells. J Virol 76:11677–11687

Hu S, Vincenz C, Buller M, Dixit VM (1997) A novel family of viral death effector domain-containing molecules that inhibit both CD-95- and tumor necrosis factor receptor-1-induced apoptosis. J Biol Chem 272:9621–9624

Javahery R, Khachi A, Lo K, Zenzie-Gregory B, Smale ST (1994) DNA sequence requirements for transcriptional initiator activity in mammalian cells. Mol Cell Biol 14:116–127

Jenner RG, Alba MM, Boshoff C, Kellam P (2001a) Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. J Virol 75:891–902

Jenner RG, Alba MM, Boshoff C, Kellam P (2001b) Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. J Virol 75:891–902

Jeong JH, Hines-Boykin R, Ash JD, Dittmer DP (2002) Tissue specificity of the Kaposi's sarcoma-associated herpesvirus latent nuclear antigen (LANA/orf73) promoter in transgenic mice. J Virol 76:11024–11032

Jeong J, Papin J, Dittmer D (2001) Differential regulation of the overlapping Kaposi's sarcoma-associated herpesvirus vGCR (orf74) and LANA (orf73) promoters. J Virol 75:1798–1807

Jeong JH, Orvis J, Kim JW, McMurtrey CP, Renne R, Dittmer DP (2004) Regulation and autoregulation of the promoter for the latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus. J Biol Chem 279:16822–16831

Kardassis D, Papakosta P, Pardali K, Moustakas A (1999) c-Jun transactivates the promoter of the human p21(WAF1/Cip1) gene by acting as a superactivator of the ubiquitous transcription factor Sp1. J Biol Chem 274:29572–29581

Katano H, Sato Y, Kurata T, Mori S, Sata T (1999) High expression of HHV-8-encoded ORF73 protein in spindle-shaped cells of Kaposi's sarcoma. Am J Pathol 155:47–52

Katano H, Sato Y, Kurata T, Mori S, Sata T (2000) Expression and localization of human herpesvirus 8-encoded proteins in primary effusion lymphoma, Kaposi's sarcoma, and multicentric Castleman's disease. Virology 269:335–344

Kedes D, H. , Lagunoff M, Renne R, Ganem D (1997a) Identification of the gene encoding the major latency-associated nuclear antigen of the Kaposi's sarcoma-associated herpesvirus. Journal of Clinical Investigation 100:2606–2610

Kedes DH, Lagunoff M, Renne R, Ganem D (1997b) Identification of the gene encoding the major latency-associated nuclear antigen of the Kaposi's sarcoma-associated herpesvirus. J Clin Invest 100:2606–2610

Kellam P et al. (1999) Characterization of monoclonal antibodies raised against the latent nuclear antigen of human herpesvirus 8. J Virol 73:5149–5155

Kellam P, Boshoff C, Whitby D, Matthews S, Weiss RA, Talbot SJ (1997a) Identification of a major latent nuclear antigen, LNA-1, in the human herpesvirus 8 genome. Journal of human virology 1:19–29

Kellam P, Boshoff C, Whitby D, Matthews S, Weiss RA, Talbot SJ (1997b) Identification of a major latent nuclear antigen, LNA-1, in the human herpesvirus 8 genome. J Hum Virol 1:19–29

Keller SA, Schattner EJ, Cesarman E (2000) Inhibition of NF-kappaB induces apoptosis of KSHV-infected primary effusion lymphoma cells. Blood 96:2537–2542

Kelley-Clarke B et al. (2007b) Determination of Kaposi's Sarcoma-Associated Herpesvirus C-Terminal Latency-Associated Nuclear Antigen Residues Mediating Chromosome Association and DNA Binding. J Virol 81:4348–4356

Kelley-Clarke B, Ballestas ME, Komatsu T, Kaye KM (2007a) Kaposi's sarcoma herpesvirus C-terminal LANA concentrates at pericentromeric and peri-telomeric regions of a subset of mitotic chromosomes. Virology 357:149–157

Kliche S et al. (2001) Signaling by human herpesvirus 8 kaposin A through direct membrane recruitment of cytohesin-1. Mol Cell 7:833–843

Krishnan HH, Naranatt PP, Smith MS, Zeng L, Bloomer C, Chandran B (2004) Concurrent expression of latent and a limited number of lytic genes with immune modulation and antiapoptotic function by Kaposi's sarcoma-associated herpesvirus early during infection of primary endothelial and fibroblast cells and subsequent decline of lytic gene expression. J Virol 78:3601–3620

Krithivas A, Fujimuro M, Weidner M, Young DB, Hayward SD (2002) Protein interactions targeting the latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus to cell chromosomes. J Virol 76:11596–11604

Krithivas A, Young DB, Liao G, Greene D, Hayward SD (2000) Human herpesvirus 8 LANA interacts with proteins of the mSin3 corepressor complex and negatively regulates Epstein-Barr virus gene expression in dually infected PEL cells. J Virol 74:9637–9645

Lagos D et al. (2007) Kaposi sarcoma herpesvirus-encoded vFLIP and vIRF1 regulate antigen presentation in lymphatic endothelial cells. Blood 109:1550–1558

Laman H, Coverley D, Krude T, Laskey R, Jones N (2001) Viral cyclin-cyclin-dependent kinase 6 complexes initiate nuclear DNA replication [In Process Citation]. Mol Cell Biol 21:624–635

Lan K, Kuppers DA, Robertson ES (2005a) Kaposi's sarcoma-associated herpesvirus reactivation is regulated by interaction of latency-associated nuclear antigen with recombination signal sequence-binding protein Jkappa, the major downstream effector of the Notch signaling pathway. J Virol 79:3468–3478

Lan K, Kuppers DA, Verma SC, Robertson ES (2004) Kaposi's sarcoma-associated herpesvirus-encoded latency-associated nuclear antigen inhibits lytic replication by targeting Rta: a potential mechanism for virus-mediated control of latency. J Virol 78:6585–6594

Lan K, Kuppers DA, Verma SC, Sharma N, Murakami M, Robertson ES (2005b) Induction of Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen by the lytic transactivator RTA: a novel mechanism for establishment of latency. J Virol 79:7453–7465

Lee BS, Paulose-Murphy M, Chung YH, Connlole M, Zeichner S, Jung JU (2002) Suppression of tetradecanoyl phorbol acetate-induced lytic reactivation of Kaposi's sarcoma-associated herpesvirus by K1 signal transduction. J Virol 76:12185–12199

Li H, Komatsu T, Dezube BJ, Kaye KM (2002) The Kaposi's sarcoma-associated herpesvirus K12 transcript from a primary effusion lymphoma contains complex repeat elements, is spliced, and initiates from a novel promoter. J Virol 76:11880–11888

Li M et al. (1997) Kaposi's sarcoma-associated herpesvirus encodes a functional cyclin. J Virol 71:1984–1991

Liang Y, Chang J, Lynch SJ, Lukac DM, Ganem D (2002) The lytic switch protein of KSHV activates gene expression via functional interaction with RBP-Jkappa (CSL), the target of the Notch signaling pathway. Genes Dev 16:1977–1989

Liang Y, Ganem D (2004) RBP-J (CSL) is essential for activation of the K14/vGPCR promoter of Kaposi's sarcoma-associated herpesvirus by the lytic switch protein RTA. J Virol 78:6818–6826

Lim C, Gwack Y, Hwang S, Kim S, Choe J (2001) The transcriptional activity of cAMP response element-binding protein-binding protein is modulated by the latency associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus. J Biol Chem 276:31016–31022

Lim C, Sohn H, Gwack Y, Choe J (2000) Latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) binds ATF4/CREB2 and inhibits its transcriptional activation activity. J Gen Virol 81:2645–2652

Lovec H, Sewing A, Lucibello FC, Muller R, Moroy T (1994) Oncogenic activity of cyclin D1 revealed through cooperation with Ha- ras: link between cell cycle control and malignant transformation. Oncogene 9:323–326

Low W, Harries M, Ye H, Du MQ, Boshoff C, Collins M (2001) Internal ribosome entry site regulates translation of Kaposi's sarcoma-associated herpesvirus FLICE inhibitory protein. J Virol 75:2938–2945.

Lu F, Day L, Gao SJ, Lieberman PM (2006) Acetylation of the latency-associated nuclear antigen regulates repression of Kaposi's sarcoma-associated herpesvirus lytic transcription. J Virol 80:5273–5282

Lu F, Zhou J, Wiedmer A, Madden K, Yuan Y, Lieberman PM (2003) Chromatin remodeling of the Kaposi's sarcoma-associated herpesvirus ORF50 promoter correlates with reactivation from latency. J Virol 77:11425–11435

Lu M et al. (2004) Dissection of the Kaposi's sarcoma-associated herpesvirus gene expression program by using the viral DNA replication inhibitor cidofovir. J Virol 78:13637–13652

Lubyova B, Pitha PM (2000) Characterization of a novel human herpesvirus 8-encoded protein, vIRF-3, that shows homology to viral and cellular interferon regulatory factors. J Virol 74:8194–8201.

Ma C, Papermaster D, Cepko CL (1998) A unique pattern of photoreceptor degeneration in cyclin D1 mutant mice. Proc Natl Acad Sci U S A 95:9938–9943

Mann DJ, Child ES, Swanton C, Laman H, Jones N (1999) Modulation of p27(Kip1) levels by the cyclin encoded by Kaposi's sarcoma-associated herpesvirus. Embo J 18:654–663

Marshall V et al. (2007) Conservation of Virally Encoded MicroRNAs in Kaposi Sarcoma-Associated Herpesvirus in Primary Effusion Lymphoma Cell Lines and in Patients with Kaposi Sarcoma or Multicentric Castleman Disease. J Infect Dis 195:645–659

Matsumura S, Fujita Y, Gomez E, Tanese N, Wilson AC (2005) Activation of the Kaposi's sarcoma-associated herpesvirus major latency locus by the lytic switch protein RTA (ORF50). J Virol 79:8493–8505

Mattsson K et al. (2002) Latent nuclear antigen of Kaposi's sarcoma herpesvirus/human herpesvirus-8 induces and relocates RING3 to nuclear heterochromatin regions. J Gen Virol 83:179–188

McAllister SC, Hansen SG, Messaoudi I, Nikolich-Zugich J, Moses AV (2005) Increased efficiency of phorbol ester-induced lytic reactivation of Kaposi's sarcoma-associated herpesvirus during S phase. J Virol 79:2626–2630

McCormick C, Ganem D (2005) The kaposin B protein of KSHV activates the p38/MK2 pathway and stabilizes cytokine mRNAs. Science 307:739–741

McCormick C, Ganem D (2006) Phosphorylation and function of the kaposin B direct repeats of Kaposi's sarcoma-associated herpesvirus. J Virol 80:6165–6170

Medema JP et al. (1997) FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). Embo J 16:2794–2804

Mesri EA et al. (1996) Human herpesvirus-8/Kaposi's sarcoma-associated herpesvirus is a new transmissible virus that infects B cells. J Exp Med 183:2385–2390

Micheletti F et al. (2002) Identification of cytotoxic T lymphocyte epitopes of human herpesvirus 8. Immunology 106:395–403

Moses AV et al. (1999) Long-term infection and transformation of dermal microvascular endothelial cells by human herpesvirus 8. J Virol 73:6892–6902

Muralidhar S et al. (1998) Identification of kaposin (open reading frame K12) as a human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) transforming gene [published erratum appears in J Virol 1999 Mar;73(3):2568]. J Virol 72:4980–4988

Mutlu AD et al. (2007) In Vivo-Restricted and Reversible Malignancy Induced by Human Herpesvirus-8 KSHV: A Cell and Animal Model of Virally Induced Kaposi's Sarcoma. Cancer Cell 11:245–258

Muzio M et al. (1996) FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death–inducing signaling complex. Cell 85:817–827

Nakamura H, Lu M, Gwack Y, Souvlis J, Zeichner SL, Jung JU (2003) Global changes in Kaposi's sarcoma-associated virus gene expression patterns following expression of a tetracycline-inducible Rta transactivator. J Virol 77:4205–4220

Neipel F, Albrecht JC, Fleckenstein B (1997) Cell-homologous genes in the Kaposi's sarcoma-associated rhadinovirus human herpesvirus 8: determinants of its pathogenicity? J Virol 71:4187–4192

Ojala PM et al. (1999) Kaposi's sarcoma-associated herpesvirus-encoded v-cyclin triggers apoptosis in cells with high levels of cyclin-dependent kinase 6 [In Process Citation]. Cancer Res 59:4984–4989

Ojala PM, Yamamoto K, Castanos-Velez E, Biberfeld P, Korsmeyer SJ, Makela TP (2000) The apoptotic v-cyclin-CDK6 complex phosphorylates and inactivates Bcl-2. Nat Cell Biol 2:819–825

Ottinger M, Christalla T, Nathan K, Brinkmann MM, Viejo-Borbolla A, Schulz TF (2006) Kaposi's sarcoma-associated herpesvirus LANA-1 interacts with the short variant of BRD4 and releases cells from a BRD4- and BRD2/RING3-induced G1 cell cycle arrest. J Virol 80:10772–10786

Pan H, Zhou F, Gao SJ (2004) Kaposi's sarcoma-associated herpesvirus induction of chromosome instability in primary human endothelial cells. Cancer Res 64: 4064–4068

Parravicini C et al. (2000) Differential viral protein expression in Kaposi's sarcoma-associated herpesvirus-infected diseases: Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. American Journal of Pathology 156:743–749

Paulose-Murphy M et al. (2001a) Transcription program of human herpesvirus 8 (kaposi's sarcoma-associated herpesvirus). J Virol 75:4843–4853.

Paulose-Murphy M et al. (2001b) Transcription program of human herpesvirus 8 (kaposi's sarcoma-associated herpesvirus). J Virol 75:4843–4853

Pearce M, Matsumura S, Wilson AC (2005) Transcripts encoding K12, v-FLIP, v-cyclin, and the microRNA cluster of Kaposi's sarcoma-associated herpesvirus originate from a common promoter. J Virol 79:14457–14464

Petre CE, Sin SH, Dittmer DP (2007) Functional p53 signaling in Kaposi's sarcoma-associated herpesvirus lymphomas: implications for therapy. J Virol 81:1912–1922

Pfeffer S et al. (2005) Identification of microRNAs of the herpesvirus family. Nat Methods 2:269–276

Platt G, Carbone A, Mittnacht S (2002) p16INK4a loss and sensitivity in KSHV associated primary effusion lymphoma. Oncogene 21:1823–1831

Platt GM, Simpson GR, Mittnacht S, Schulz TF (1999) Latent nuclear antigen of Kaposi's sarcoma-associated herpesvirus interacts with RING3, a homolog of the Drosophila female sterile homeotic (fsh) gene. J Virol 73:9789–9795

Radkov SA, Kellam P, Boshoff C (2000a) The latent nuclear antigen of Kaposi sarcoma-associated herpesvirus targets the retinoblastoma-E2F pathway and with the oncogene Hras transforms primary rat cells. Nat Med 6:1121–1127

Radkov ST, Kellam P, Boshoff C (2000b) The latent nuclear antigen of Kaposi sarcoma-associated herpesvirus targets the retinoblastoma–E2F pathway and with the oncogene Hras transforms primary rat cells. Nature Medicine 6:1121–1127

Rainbow L et al. (1997) The 222- to 234-kilodalton latent nuclear protein (LNA) of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) is encoded by orf73 and is a component of the latency-associated nuclear antigen. J Virol 71:5915–5921

Renne R, Barry C, Dittmer D, Compitello N, Brown PO, Ganem D (2001) Modulation of cellular and viral gene expression by the latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus. J Virol 75:458–468

Rivas C, Thlick AE, Parravicini C, Moore PS, Chang Y (2001a) Kaposi's sarcoma-associated herpesvirus LANA2 is a B-cell-specific latent viral protein that inhibits p53. J Virol 75:429–438.

Rivas C, Thlick AE, Parravicini C, Moore PS, Chang Y (2001b) Kaposi's sarcoma-associated herpesvirus LANA2 is a B-cell-specific latent viral protein that inhibits p53. J Virol 75:429–438

Russo James J et al. (1996b) Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). Proceedings of the National Academy of Science USA 93:14862–14867

Russo JJ et al. (1996a) Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). Proc Natl Acad Sci U S A 93:14862–14867

Sadler R et al. (1999) A complex translational program generates multiple novel proteins from the latently expressed kaposin (K12) locus of Kaposi's sarcoma- associated herpesvirus. J Virol 73:5722–5730

Samols MA, Hu J, Skalsky RL, Renne R (2005) Cloning and identification of a microRNA cluster within the latency-associated region of Kaposi's sarcoma-associated herpesvirus. J Virol 79:9301–9305

Sarek G et al. (2007) Reactivation of the p53 pathway as a treatment modality for KSHV-induced lymphomas. J Clin Invest 117:1019–1028

Sarid R, Flore O, Bohenzky RA, Chang Y, Moore PS (1998) Transcription mapping of the Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) genome in a body cavity-based lymphoma cell line (BC-1). J Virol 72:1005–1012

Sarid R, Olsen SJ, Moore PS (1999c) Kaposi's sarcoma-associated herpesvirus: epidemiology, virology, and molecular biology. Adv Virus Res 52:139–232

Sarid R, Wiezorek JS, Moore PS, Chang Y (1999a) Characterization and cell cycle regulation of the majorKaposi's sarcoma-associated herpesvirus (human herpesvirus 8) latent genes and their promoter. J Virol 73:1438–1446

Sarid R, Wiezorek JS, Moore PS, Chang Y (1999b) Characterization and cell cycle regulation of the major Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) latent genes and their promoter. J Virol 73:1438–1446

Schmittgen TD, Jiang J, Liu Q, Yang L (2004) A high-throughput method to monitor the expression of microRNA precursors. Nucleic Acids Res 32:e43

Shamay M, Krithivas A, Zhang J, Hayward SD (2006) Recruitment of the de novo DNA methyltransferase Dnmt3a by Kaposi's sarcoma-associated herpesvirus LANA. Proc Natl Acad Sci U S A

Sherr CJ (1996) Cancer cell cycles. Science 274:1672–1677

Shinohara H et al. (2002) Chromosome binding site of latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus is essential for persistent episome maintenance and is functionally replaced by histone H1. J Virol 76:12917–12924

Si H, Robertson ES (2006) Kaposi's sarcoma-associated herpesvirus-encoded latency-associated nuclear antigen induces chromosomal instability through inhibition of p53 function. J Virol 80:697–709

Si H, Verma SC, Robertson ES (2006) Proteomic analysis of the Kaposi's sarcoma-associated herpesvirus terminal repeat element binding proteins. J Virol 80:9017–9030

Sin SH et al. (2007) Rapamycin is efficacious against primary effusion lymphoma (PEL) cell lines in vivo by inhibiting autocrine signaling. Blood 109:2165–2173

Skapek SX et al. (2001) Persistent expression of cyclin D1 disrupts normal photoreceptor differentiation and retina development. Oncogene 20:6742–6751

Smale ST (1997) Transcription initiation from TATA-less promoters within eukaryotic protein-coding genes. Biochim Biophys Acta 1351:73–88

Smale ST (2001) Core promoters: active contributors to combinatorial gene regulation. Genes Dev 15:2503–2508

Srinivasan V, Komatsu T, Ballestas ME, Kaye KM (2004) Definition of sequence requirements for latency-associated nuclear antigen 1 binding to Kaposi's sarcoma-associated herpesvirus DNA. J Virol 78:14033–14038

Staskus KA et al. (1997) Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells. J Virol 71:715–719

Staskus KA et al. (1999) Cellular tropism and viral interleukin-6 expression distinguish human herpesvirus 8 involvement in Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. J Virol 73:4181–4187

Staudt MR, Dittmer DP (2006) Promoter switching allows simultaneous transcription of LANA and K14/vGPCR of Kaposi's sarcoma-associated herpesvirus. Virology 350:192–205

Staudt MR, Kanan Y, Jeong JH, Papin JF, Hines-Boykin R, Dittmer DP (2004) The tumor microenvironment controls primary effusion lymphoma growth in vivo. Cancer Res 64:4790–4799

Stedman W, Deng Z, Lu F, Lieberman PM (2004) ORC, MCM, and histone hyperacetylation at the Kaposi's sarcoma-associated herpesvirus latent replication origin. J Virol 78:12566–12575

Suscovich TJ et al. (2004) Defective immune function of primary effusion lymphoma cells is associated with distinct KSHV gene expression profiles. Leuk Lymphoma 45:1223–1238

Swanton C, Mann DJ, Fleckenstein B, Neipel F, Peters G, Jones N (1997) Herpes viral cyclin/Cdk6 complexes evade inhibition by CDK inhibitor proteins. Nature 390:184–187

Szekely L et al. (1999) Human herpesvirus-8-encoded LNA-1 accumulates in heterochromatin- associated nuclear bodies. J Gen Virol 80(Pt 11):2889–2900

Talbot SJ, Weiss RA, Kellam P, Boshoff C (1999) Transcriptional analysis of human herpesvirus-8 open reading frames 71, 72, 73, K14, and 74 in a primary effusion lymphoma cell line. Virology 257:84–94

Upton JW, Speck SH (2006) Evidence for CDK-dependent and CDK-independent functions of the murine gammaherpesvirus 68 v-cyclin. J Virol 80:11946–11959

van Dyk LF, Hess JL, Katz JD, Jacoby M, Speck SH, Virgin HI (1999) The murine gammaherpesvirus 68 v-cyclin gene is an oncogene that promotes cell cycle progression in primary lymphocytes. J Virol 73:5110–5122

Verma SC, Bajaj BG, Cai Q, Si H, Seelhammer T, Robertson ES (2006a) Latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus recruits uracil DNA glycosylase 2 at the terminal repeats and is important for latent persistence of the virus. J Virol 80:11178–11190

Verma SC, Borah S, Robertson ES (2004) Latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus up-regulates transcription of human telomerase reverse transcriptase promoter through interaction with transcription factor Sp1. J Virol 78:10348–10359

Verma SC, Choudhuri T, Kaul R, Robertson ES (2006b) Latency-associated nuclear antigen (LANA) of Kaposi's sarcoma-associated herpesvirus interacts with origin recognition complexes at the LANA binding sequence within the terminal repeats. J Virol 80:2243–2256

Verma SC, Lan K, Choudhuri T, Robertson ES (2006c) Kaposi's sarcoma-associated herpesvirus-encoded latency-associated nuclear antigen modulates K1 expression through its cis-acting elements within the terminal repeats. J Virol 80:3445–3458

Verschuren EW, Hodgson JG, Gray JW, Kogan S, Jones N, Evan GI (2004) The role of p53 in suppression of KSHV cyclin-induced lymphomagenesis. Cancer Res 64:581–589

Verschuren EW, Klefstrom J, Evan GI, Jones N (2002) The oncogenic potential of Kaposi's sarcoma-associated herpesvirus cyclin is exposed by p53 loss in vitro and in vivo. Cancer Cell 2:229–241

Viejo-Borbolla A et al. (2005) Brd2/RING3 interacts with a chromatin-binding domain in the Kaposi's Sarcoma-associated herpesvirus latency-associated nuclear antigen 1 (LANA-1) that is required for multiple functions of LANA-1. J Virol 79:13618–13629

Wang D et al. (2002) Microarray-based detection and genotyping of viral pathogens. Proc Natl Acad Sci U S A 99:15687–15692

Weis L, Reinberg D (1992) Transcription by RNA polymerase II: initiator-directed formation of transcription-competent complexes. Faseb J 6:3300–3309

Whitby D et al. (2007) Reactivation of Kaposi's sarcoma-associated herpesvirus by natural products from Kaposi's sarcoma endemic regions. Int J Cancer 120:321–328

Wong LY, Matchett GA, Wilson AC (2004) Transcriptional activation by the Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen is facilitated by an N-terminal chromatin-binding motif. J Virol 78:10074–10085

Ye FC, Zhou FC, Yoo SM, Xie JP, Browning PJ, Gao SJ (2004) Disruption of Kaposi's sarcoma-associated herpesvirus latent nuclear antigen leads to abortive episome persistence. J Virol 78:11121–11129

Yi R, Doehle BP, Qin Y, Macara IG, Cullen BR (2005) Overexpression of exportin 5 enhances RNA interference mediated by short hairpin RNAs and microRNAs. Rna 11:220–226

Yoo SM, Zhou FC, Ye FC, Pan HY, Gao SJ (2005) Early and sustained expression of latent and host modulating genes in coordinated transcriptional program of KSHV productive primary infection of human primary endothelial cells. Virology 343:47–64

Yuan J, Cahir-McFarland E, Zhao B, Kieff E (2006) Virus and cell RNAs expressed during Epstein-Barr virus replication. J Virol 80:2548–2565

Zeng Y, Yi R, Cullen BR (2005) Recognition and cleavage of primary microRNA precursors by the nuclear processing enzyme Drosha. Embo J 24:138–148

Zhang YJ, Deng JH, Rabkin C, Gao SJ (2000) Hot-spot variations of Kaposi's sarcoma-associated herpesvirus latent nuclear antigen and application in genotyping by PCR-RFLP. J Gen Virol 81:2049–2058

Zhong W, Wang H, Herndier B, Ganem D (1996) Restricted expression of Kaposi sarcoma-associated herpesvirus (human herpesvirus 8) genes in Kaposi sarcoma. Proc Natl Acad Sci U S A 93:6641–6646

Chapter 20

Query No.	Line No.	Query
AQ1	261	Please specify the year for reference citation "Dittmer et al.".
AQ2	264	Please specify the year for reference citation "Hu et al."
AQ3	313	Please specify the year for the reference citation "Verma et al. through out the chapters.
AQ4	324	Please specify the exact year for the reference citation Jeong et al.
AQ5	557	Please provide the chapter number in the sentence "Their function ..."
AQ6	571	Please check the alignment of "Table 20.3"