Characterization and Cell Cycle Regulation of the Major Kaposi’s Sarcoma-Associated Herpesvirus (Human Herpesvirus 8) Latent Genes and Their Promoter

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Kaposi’s sarcoma-associated herpesvirus (KSHV or HHV8) is a gammaherpesvirus (9, 30) etiologically linked to Kaposi’s sarcoma (KS), primary effusion lymphoma (PEL, also termed body cavity-based lymphoma [BCBL]), and a subset of multicentric Castleman’s disease (6, 9, 32, 42). Herpesviruses establish latent infections in their natural hosts characterized by persistence of the viral genome as a covalently closed, circular episome with limited viral gene expression (37). Most cells infected with KSHV demonstrate limited in situ viral gene expression consistent with virus latency (12, 33, 43). Tissue culture B-cell lines derived from PELs also have limited viral gene expression, although in some lines a minority population of cells can undergo spontaneous lytic replication (27, 35, 48). TPA (12-O-tetradecanoylphorbol-13-acetate) or sodium butyrate treatment of PEL cell lines has been divided into three classes based on immortalization of infected cells (23). KSHV transcription in PEL cell lines has been divided into three classes based on responsiveness to phorbol ester treatment, class I transcripts are expressed constitutively but are also induced by TPA treatment, and class III transcripts are only expressed after TPA induction corresponding to late lytic gene expression. A survey of KSHV gene transcription in BC-1 cells demonstrated that most KSHV genes are readily inducible by TPA treatment (class II or III transcripts). Two transcripts encoded on the right-hand end of the genome, however, show equal abundance in TPA-treated and untreated BC-1 cells, consistent with their designation as latent (class I) transcripts (40). Preliminary mapping of these transcripts indicated that the 6.0-kb transcript, designated latent transcript 1 (LT1), encodes ORF73, ORF72 (vCYC), and ORF K13 (vFLIP), while the 2.0-kb transcript, designated LT2, only encodes ORF73 and ORF K13 but not ORF72. Expression of ORF73-containing partial cDNA clones has demonstrated that this gene encodes the latency-associated nuclear antigen (LANA) (20, 33), an antigen target for several serological assays (14). In addition to the constitutively expressed latent transcripts LT1 and LT2, other class I KSHV transcripts in PEL cell lines include LT3 (40) and a transcript from the g block region of the KSHV genome that was not identified during the whole genome transcriptional mapping survey of Sarid et al. (9a, 40).

Characterization of LT1 and LT2 expression may provide important information on KSHV and its interaction with the host cell. vCYC, the protein product of the ORF72 gene, has been shown to be a functional cyclin capable of directing phosphorylation and inactivation of the retinoblastoma tumor suppressor protein (pRB) as well as phosphorylation of histone H1 (10, 15, 25). Mechanisms to inhibit pRB are a common feature of tumor viruses (for a review see reference 29). Expression of vCYC during latency suggests that KSHV has the capacity to independently regulate host cell transit through the pRB-controlled G1 checkpoint. Similarly, the vFLIP encoded by ORF K13 (sometimes referred to as ORF71) on the LT transcripts is likely to have anti-apoptotic activity that might...
affect survival of infected cells and contribute to the tumor phenotype (3). Thus, determining the expression patterns of the LT transcripts may delineate how KSHV affects cellular proliferation in tumors. Two considerations, however, are important for interpreting this data. First, posttranscriptional regulation is also likely to modulate expression of some latent proteins, such as vFLIP (32a). Second, KSHV shows evidence of tissue-specific transcriptional control such that results from studies of cultured cell lines may differ from tumors studied in situ. Cell culture studies, however, allow direct manipulation and increased discrimination of viral gene expression.

In this study, we examined the KSHV LT1 and LT2 transcripts by cDNA cloning, 5′ rapid amplification of cDNA ends (5′ RACE), and primer extension analyses. The promoter region for LT1 and LT2 (LP1/2) was examined with deletion reporter constructs to determine its transcriptional regulation. These studies also demonstrate that the LP1/2 promoter is activated in a cell cycle-dependent manner, which is one of the few examples of a viral gene regulated by the cell cycle. KSHV vCYC may supplement or substitute for downregulated cellular D cyclin activity to maintain cell cycle periodicity. Expression of the KSHV vCYC during latency and its regulation by the cell cycle is consistent with the virus attempting to reestablish cell cycle homeostasis in the setting of increased expression of the G1 checkpoint to limit latent virus replication (29).

### MATERIALS AND METHODS

**Cells.** BC-1 (PEL-derived B-cell line infected with KSHV and Epstein-Barr virus [EBV]) (7), BCBL-1 (PEL-derived B-cell line infected with KSHV only) (35), P3HR1 (B-cell line infected with EBV), and BJAB (KSHV and EBV virus [EBV]) (7), BCBL-1 (PEL-derived B-cell line infected with KSHV only) were maintained at 37°C in RPMI 1640 medium (GibcoBRL, Gaithersburg, Md.) containing 2 mM l-glutamine and 10% fetal calf serum (FCS) (GibcoBRL) in the presence of 5% CO2. To induce lytic gene transcription, cells were exposed to 20 ng of TPA (Sigma Chemical Co., St. Louis, Mo.)/ml and harvested after 48 h (30). To inhibit virus DNA replication, phosphonoformic acid {PAMPA} (Calbiochem, La Jolla, Calif.) was added at 200 mM final concentration and harvested for flow cytometry and RNA extraction at 0, 3, 6, 10, and 24 h after exposure with the ORF72 promoter was used to standardize loading.

**Construction and screening of BC-1 cell line cDNA library.** A cDNA phage library of BC-1 cell line was constructed in the ZAP ExpressTM vector (Stratagen, La Jolla, Calif.). Clones identified with the ORF72 (vCYC) probe were plaque purified; positive plagues were converted into phagemids by employing the ExcisHelp helper phage (Strategene), and inserts were sequenced by automated DNA sequencing (ABl 377 Sequenator; Perkin-Elmer, Foster City, Calif.) with specific internal primers. 5′ RACE. 5′ rapid amplification of cDNA ends (5′ RACE) was performed by using the Marathon TM cDNA amplification kit (Clontech, Palo Alto, Calif.). To overcome difficulties in reverse transcription because of high GC content and resistant to reverse transcription, we synthesized first strand cDNA by using M13 forward primer and avian myeloblastosis virus reverse transcriptase (Promega) at 35°C. The RACE primers RPI (nucleotide [nt] 123598 to 123691), 5′-AGAGGCTCTGATTTAGGTGC-3′, and RP2 (nt 128830 to 128849), 5′-CCCGCC GAGTCTGTTTTC-3′ and an adapter primer were used for specific cDNA PCR amplification.

**Plasmids.** Reporter gene plasmids were constructed by ligating LP1/2 fragments into the EcoRI and Xhol sites of pGL3-basic vector, which contains a promotorless luciferase gene downstream of the cloning site (Promega). Reaction products were precipitated by the addition of 2.5 volumes of ethanol and resolved on a 6% polyacrylamide–7 M urea gel in Tris-borate-EDTA. Parallel DNA sequencing reactions were performed with Sequenase 2.0 (United States Biochemical Corporation, Cleveland, Ohio). pGEM template sequence with the M13 forward primer was used as a size marker.

**RESULTS**

**Northern blot analysis.** Probes internal to ORF72 (vCYC) (Fig. 1A) and ORF K13 (vFLIP) (Fig. 1C) detect 6.0- and 2.0-kb bands corresponding to LT1 and LT2 transcripts, respectively, as previously reported (40). In contrast, the ORF73 (LANA) probe only detects the 6.0-kb LT1 transcript. Prolonged exposure with the ORF72 probe also demonstrates the presence of a low-abundance transcript approximately 1 kb in

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size that does not hybridize with either the ORF K13 or ORF73 probe (Fig. 1A). We were unable to further characterize this small transcript due to its low abundance. As previously demonstrated (13, 40), the LT1 and LT2 transcripts are not induced by TPA treatment nor are they inhibited by treatment with the DNA polymerase inhibitor PFA (Fig. 1A to C). In comparison, expression of the 7.0-kb ORF25 transcript is both induced by TPA and inhibited by PFA treatment, which is consistent with its designation as a late lytic-phase gene (Fig. 1D).

Transcript mapping. To characterize LT1 and LT2, we screened approximately 2 × 10⁵ clones from a BC-1 cDNA library with a radiolabeled ORF72 PCR probe. Approximately 30 positive clones were identified, and 8 were isolated for plasmid excision and sequencing. cDNA inserts ranged from 1.4 to 1.8 kb, and each possessed a poly(A) tail with a conserved polyadenylation signal, AAUAAA (24 to 33 bp from the 3′ terminus), at a position corresponding to nt 122,093 in the BC-1 sequence (39). While most of the cDNA inserts were found to be prematurely 5′ truncated, three cDNAs provided information on transcript splicing patterns. Two cDNAs, φ1 and φ3, are full-length (or near full-length, see below) cDNAs of the LT2 transcript (Fig. 2). Both are spliced and bicistronic, encoding ORF K13, ORF72, and a short, noncoding 58-bp exon 5′ to the ORF73 gene. The intervening region (nt 123,775 to 127,812), including ORF73, between ORF72 and the 5′ exon, is spliced out from these two inserts at conserved splice donor (5′-ATAAAACA-GTGAGTA-3′) and acceptor (5′-TTGTCAG-ACCAAGCCAC-3′) sequences (4). Both φ1 and φ3 have the same 5′ ends corresponding to nt 127,870. A third cDNA clone, φ15, was the only cDNA from the eight cDNAs isolated which corresponded to the LT1 transcript (Fig. 2). It is a truncated cDNA which is colinear to genomic DNA throughout its length (i.e., it does not possess the splice site present in LT2) and contains ORF K13 and ORF72 as well as a portion of the carboxy-terminal coding sequence of ORF73, indicating that all three genes are expressed on the LT1 transcript.

5′ RACE was performed on cDNA reverse-transcribed poly(A)-selected BC-1 RNA to further delineate splicing patterns for LT1 and LT2. Two primers were designed to preferentially amplify either the LT1 or LT2 cDNA. The 5′ RACE primer 1 (RP1) (Fig. 2), located immediately 3′ to the splice acceptor site, at nt 123,775, exclusively amplified a 235-bp PCR product corresponding to the LT2 transcript (data not shown). The RP1 PCR product had the same 5′ end (nt 127,870) and splice junction (nt 123,775 to 127,812) as that found in the cDNA φ1 and φ3 LT2 inserts. This product is consistent with preferential amplification of only the spliced LT2 cDNA by the RP1 primer. The RP2 primer, located immediately 5′ to the splice acceptor site at nt 123,775 (within the LT2 intron), amplified a 3,542-bp product corresponding to the LT1 transcript. The sequence of the RP2 5′ RACE product demonstrated that LT1 also has a spliced intron sequence that uses the same splice donor site as LT2 (nt 127,812) but the splice acceptor site is at nt 127,314, corresponding to a second conserved splice acceptor sequence (5′-TTGTCAG-ACCAAGCAT-3′). The RP2 5′ RACE product also had the same 5′ end at nt 127,870 as the RP1 product.

To confirm the authenticity of the transcription start sites determined by 5′ RACE and cDNA sequencing, we performed primer extension analyses. Primer extension primer 1 (PE1) was designed to anneal to a 3′ site (nt 127,817) located 54 bp 3′ to the putative transcriptional start site determined by 5′ RACE at nt 127,870. The PE1 primer (Fig. 2) synthesized two oligonucleotide products (Fig. 3), which extends the presumed start site(s) beyond the site determined by 5′ RACE and cDNA sequencing. The major transcriptional start site for both LT1 and LT2 transcripts is a unique adenine at nt 127,900 located 30 bp beyond the start site determined by cDNA sequencing and 5′ RACE. A second minor start site is present at
nt 127,948, which is usually less intense than the nt 127,900 product on repeated analysis. A laddering pattern (Fig. 3) is present 47 to 54 bp from the PE1 primer which corresponds to the nt 127,870 “start” site found by cDNA and 5' RACE analyses. This is consistent with premature termination of the reverse transcriptase reaction and is the likely explanation for the shorter products found by these methods. The primer extension results for PE1 shown in Fig. 3 are atypical in that the minor start site at nt 127,948 (Fig. 3, 132 bp upstream of the PE1 primer) is more intense than the major start site at nt 127,900 (Fig. 3, 84 bp upstream of the PE1 primer) but the figure is shown to highlight the laddering pattern occurring at nt 127,870 (Fig. 3, 47 to 54 bp upstream of the PE1 primer). These start sites were confirmed for the LT1 transcript by using a primer (PE3) located within the LT2 intron at nt 127,268 and for the LT2 transcript by using a primer (PE2) located 3' to the splice acceptor site at nt 123,745. For both transcripts, transcription was predominantly initiated at nt 127,900.

Taken together, the cDNA sequencing, 5' RACE, and primer extension analyses indicate that LT1 and LT2 are overlapping, alternatively spliced, and polycistronic transcripts (Fig. 2). The LT1 transcript (i) corresponds to the 6.0-kb mRNA, (ii) encodes ORF K13, ORF72, and ORF73, (iii) has a major transcriptional start site at nt 127,900 and a minor transcriptional start site at nt 127,948, and (iv) uses a splice junction with the splice donor site at nt 127,812 (5'-ATAAAA CAAGTGAGTA-3') and a splice acceptor site at nt 127,314 (5'-TTTATGACGACCAGAT-3'). In contrast, the LT2 transcript (i) corresponds to the 2.0-kb mRNA, (ii) encodes ORF K13 and ORF72 but not ORF73, (iii) has an identical 3' end to LT1 and shares the same major 5' start site, (iv) uses the same splice donor site as LT1 at nt 127,812 but has a splice acceptor sequence at nt 123,775 (5'-TCCCTAGAAGCCAC-3'), which splices the ORF73 gene out of the LT2 transcript.

Analysis of the promoter sequence. An 842-bp sequence encompassing the LP region for LT1 and LT2 (LP1/2) was examined for potential regulatory sites (Fig. 4). LP1/2 possesses a noncanonical TATA box 34 bp upstream of the nt 127,900 mRNA transcription start site and a CAAT element 15 bp upstream of the putative TATA box. Additionally, two conserved initiator (Inr) elements, TATCATTT (11 to 20 bp) and CTCCACTA (220 to 28 bp), flank the major transcription start site. Inr elements are present in TATA-containing promoters as well as those that lack a confirmed TATA box (38). Inr elements bind the transcription factor TFIID and can replace a TATA box as a site to initiate RNA polymerase II-dependent transcription. In this case, transcriptional initiation is less precisely positioned and occurs at a cluster of start points (5, 26).

A search of the LP1/2 region for potential transcription factor binding sites identified an Sp1 binding site located 3' to the TATA box and immediately 5' to one of the Inr sites. Several more conserved Sp1 binding sites are clustered around bp -350. Conserved Oct-1 and Oct-6 binding sites were also found (Fig. 4). Oct-1 is a ubiquitous transcription factor that recognizes an octamer sequence also recognized by Oct-2, a lymphoid-specific transcription factor. Two conserved interferon regulatory factor (IRF1/2) binding sites and a semiconserved interferon-stimulated response element (ISGF3) are present, which suggests the promoter may be regulated by interferon signal transduction pathways as has been shown for the EBV EBNA-1 Qp promoter (31, 46). Conserved c-Jun,
c-myc, and NF1 binding sites are also present in this region. One AP-1 conserved binding site is present, although LT1-2 transcription has not been found to be responsive to TPA (13, 14, 40).

LP1/2-luciferase reporter activity. To confirm that the 5' flanking region functions as a promoter, we assessed its ability to drive expression of a reporter gene in transiently transfected cells. Promoter-reporter gene recombinants were constructed in which various lengths of the LP1/2 promoter were cloned in front of a luciferase reporter gene (pGL3.1-6) (Fig. 4). The sites of fusion were in the 5' untranslated leader sequence of LT1-2 corresponding to nt 127,870. Results from three independent experiments are shown in Fig. 5. When transfected into human epithelial HeLa cells, a construct containing sequences –774 to +30 (pGL3.6) demonstrated 10-fold greater luciferase activity over that of the basal activity of the pGL3-basic luciferase construct. Truncation of promoter length from the 5' end had little effect on promoter activity, and a minimal promoter containing 67 bp of the promoter sequence was able to initiate reporter transcription efficiently. Similar results were found when reporter plasmids were transfected into the EBV-negative B-cell line BJAB; however, luciferase activity was much higher for each promoter construct. In contrast to the experiments with HeLa cells, luciferase activity in the BJAB cell line was 32-fold higher for pGL3.6 (–774 to +30) and 58-fold higher for pGL3.4 (–378 to +30) than for pGL3-basic. The higher transcriptional activity of the reporter in BJAB compared to HeLa cells may represent lymphoid-specific transcription, while the decreased activity of pGL3.6 compared to that of pGL3.4 in BJAB cells suggests a possible lymphoid-specific repressor element in this region.

Although the presence of a conserved AP-1 (–550) suggests responsiveness to TPA, we found no evidence for enhanced reporter activity for any of the five LP1/2-luciferase constructs in HeLa (Fig. 6A) and BJAB (Fig. 6B) cells treated with 20-ng/ml TPA. TPA treatment of BJAB cells resulted in minimal inhibition of reporter activity for the pGL3.4 and pGL3.6 constructs but this may have been due to cellular toxicity rather than a direct effect of TPA-induced signaling on the AP1 promoter site. Similarly, assays with LP1/2-luciferase-reporter constructs showed no response to interferon treatment or transfection with either human IRF-1 or KSHV vIRF expression plasmids in either HeLa and BJAB cells despite the presence of potential interferon responsive elements in the promoter (not shown).

Cell cycle control of LT1-2 transcription. To examine cell cycle regulation of the LP1/2 promoter, the pGL3.6 luciferase reporter activity was compared to those of a cellular cyclin D1 (huCYC D1) promoter and a constitutively active CMV promoter in NIH 3T3 cells. Promoter-reporter constructs were transfected into NIH 3T3 cells which were then serum arrested for 0.1% FCS for 60 h. Release from serum starvation by serum stimulation for 14 h resulted in a 4-fold induction for the human cyclin D1 promoter and an 11-fold induction of the KSHV LP1/2 promoter compared to only a 1.7-fold induction for the CMV promoter (Fig. 7A). Parallel flow cytometry determinations (Fig. 7B) demonstrate that serum starvation for 60 h effectively arrests mitogenesis of NIH 3T3 cells (0% in S phase) and addition of serum for 14 h initiates cell cycle progression (32% in S phase).

Direct confirmation of KSHV LP1/2 dependence on the cell cycle was performed by Northern analysis of LT1 and LT2 expression in BCBL-1 cells (Fig. 8). Unlike NIH 3T3 cells, PEL cells are resistant to arrest by serum starvation and continue to proliferate in the absence of serum but undergo marked loss of viability, an effect similar to that seen for EBV-immortalized cells (11). Growth arrest of PEL cells, however, was achieved by 200 mM l-mimosine treatment for 20 h, which arrests cells in late G2 phase (16). l-mimosine-induced arrest is reversible by washing BCBL-1 cells with fresh medium (Fig. 9). For comparison, expression of the human cyclin D2 gene was examined because preliminary Northern analyses showed that only cyclin D2, and not cyclin D1 or D3, was appreciably expressed in BCBL-1 cell lines. KSHV LT1 and LT2 (Fig. 8A) and human cyclin D2 (Fig. 8B) expression was nearly absent in l-mimosine-arrested cells at time zero (87% G0/G1) (Fig. 9B). Expression of these transcripts progressively increased in parallel after release from l-mimosine arrest. l-mimosine arrest had no effect on GAPDH transcription used to confirm RNA loading (Fig. 8C).

DISCUSSION

Like other herpesviruses, KSHV gene transcription is limited during presumed virus latency (40, 48). KSHV latent (or class I) gene expression has been functionally defined as being constitutive in PEL tissue culture cells (40) in that it is not induced by phorbol esters nor inhibited by DNA polymerase inhibitors. In contrast, class III genes such as ORF25, which
encodes the MCP, are expressed in a manner consistent with late lytic cycle genes in that they are not expressed without TPA treatment in some highly restricted cell lines such as BC-1 (40).

In this study we defined the nucleotide sequences and gene organization of two KSHV latent transcripts, LT1 and LT2, that were found during a survey for KSHV gene transcription (40). The constitutive transcription of LT1-2 transcripts is consistent with transcriptional studies of vCYC mRNA performed with BC-1 and BC-3 cell lines (13) and for LANA protein expression with BC-1 cells (14). Both LT1 and LT2 are spliced transcripts which have the same transcriptional start site and a

FIG. 4. Nucleotide sequence of the latent promoter region (LPI/2) for LT1-2. The first nucleotide of the presumed major transcription start site at nt 127,900 is marked by a single asterisk and is indicated in boldface. The second start site is marked by a double asterisk, and potential transcription factor binding sites in the promoter region are indicated. The locations of promoter deletions used for reporter studies indicated by arrows.

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FIG. 5. Luciferase expression levels for LP1/2-luciferase reporter constructs in HeLa (white bars) and BJAB (grey bars) cells. HeLa cells were transfected with 1 mg of reporter gene and 1 mg of control plasmid pcDNA3.1/lacZ. BJAB cells were transfected with 10 mg of reporter gene and 10 μg of control plasmid pcDNA3.1/lacZ. Mean promoter activities obtained from three transfections after normalization for β-galactosidase expression are indicated relative to the pGL3-luc basic reporter plasmid lacking a promoter sequence.
splice junction beginning after a short untranslated 5' leader sequence. The LT1 intron is a short noncoding region, whereas the LT2 intron splices out the entire ORF73 gene. Our primer extension analysis suggests that an additional transcription start site is present; however, we found no evidence for differential regulation of LT1 and LT2, and a more comprehensive examination of study of transcriptional factor activation may reveal important differences in expression of these transcripts. The reasons why the KSHV ORF73 gene encoding LANA is spliced from LT2 and why the virus possesses these two overlapping transcripts can only remain speculative since little is currently known about the functions of the LANA protein.

The promoter region for these transcripts, LP1/2, was transcriptionally active in both epithelial and lymphoid cell lines by using a luciferase reporter construct. The higher activity in BJAB cells suggests transcription might be specifically enhanced in lymphoid cell lines. Although a 67-bp minimal LP1/2 promoter was sufficient to drive efficient transcription of the reporter gene, the putative promoter region contains a number of potential transcriptional factor binding sites that might regulate transcription. While examination of promoter-reporter constructs in tissue culture provides important information on LT1-2 transcriptional regulation, an important caveat is that cell type-specific transcriptional programs exist for KSHV (28, 34). Direct examination of tissue-specific transcription and translation is necessary to extend our results directly to tumors in situ.

At least two nuclear proteins, LANA (ORF73) and vCYC

FIG. 6. Effect of TPA treatment on LP1/2-luciferase promoter reporter constructs. HeLa (A) and BJAB (B) cells cultured in the presence (grey bars) or the absence (white bars) of 20-ng/ml TPA were transfected with LP1/2-luciferase constructs as described in the legend for Fig. 4 and assayed for luciferase activity. No evidence for TPA inducibility was found for either cell type.

FIG. 7. (A) Cell cycle-dependent induction of the pGL3.6 LP1/2-luciferase reporter. NIH 3T3 cells were transfected with the indicated constructs and cultured in low serum (0.1% FCS) for 60 h. Bars indicate the levels of induction of luciferase activity 14 h after readdition of 20% FCS compared to pretreatment luciferase activity for the constitutively active CMV, the human cyclin D1, and the KSHV LP1/2 promoters. (B) Cell cycle distributions as determined by FACS analyses at 0 and 14 h after serum readdition. Serum starvation for 60 h arrested 90% of NIH 3T3 cells in G0/G1, whereas 32% of cells were entering S phase 14 h after serum stimulation.

FIG. 8. Time course of KSHV vCYC (A) and cellular huCYC D2 (B) mRNA expression in BCBL-1 cells after release from t-mimosine G1 arrest. GAPDH mRNA expression (C) was used as a control for equal mRNA loading. Cells were arrested for 20 h by treatment with 200 mM t-mimosine and released from cell cycle arrest by washing in fresh medium. Cell cultures were harvested at 0, 3, 6, 10, and 24 h after t-mimosine washout and prepared for mRNA extraction. Expression of KSHV LT1 and LT2 mRNA mirrors expression of cellular CYC D2, which is expressed early in G1 phase of the cell cycle.
(ORF72) are expressed during viral latency (12, 30). LANA possesses a long acidic repeat domain which is reminiscent of a similar domain in the latent EBV EBNA-1 protein that inhibits cytotoxic T-lymphocyte recognition of this antigen (24). At present it is unclear whether LANA functions like EBNA-1 in maintaining viral episomal replication or has other functions during viral latency. No functional data are available to determine if ORF73 plays a role in cell cycle regulation or transformation; however, NIH 3T3 cells stably expressing ORF73 alone are not transformed (40a). KSHV vCYC has a high degree of sequence homology with cellular D-type cyclins (8) and is functionally similar to these cell cycle control proteins (10). vCYC can overcome the G1-S block mediated by pRb in SAOS-2 cells (10) and induce pRb and histone H1 phosphorylation via CDK6 activation (15, 25). Further, the viral cyclin, unlike cellular D-type cyclins, is resistant to p16, p21, and p27 CDK inhibitors (44). A third protein encoded by these latent transcripts, vFLIP (ORF K13), is a dominant negative inhibitor of Fas/APO1-activated apoptosis. Preliminary studies suggest that this protein may be under posttranscrip-
tional regulation and is not expressed during latency in PEL cells despite active transcription of the gene on the life cycles of both DNA and RNA tumor viruses, pRB inhibition has been attributed to the need for a virus to induce S-phase DNA synthesis in order to expand virion production (18). This is likely to be the case for at least some tumor viruses during lytic virus replication. EBV, for example, encodes lytic replication transactivator proteins (BRLF1 and BZLF1) which directly inhibit pRB and p53 proteins (45, 47). However, pRB inactivation during virus latency does not preferentially replicate viral genome over host cell genome. An alternative explanation for pRB inhibition during virus latency is that cell cycle arrest serves as an antiviral mechanism to limit latent virus replication (29). Our data is consistent with this latter hypothesis that the KSHV vCYC is expressed during virus latency. Rather than being constitutively overexpressed, vCYC transcription appears to be closely regulated by cell cycle-specific signaling events. Swanton and colleagues have demonstrated that the KSHV vCYC is resistant to cellular CDK inhibitors that control D cyclin activities (44). This suggests that KSHV replaces cellular D cyclin activity with a viral cyclin that is resistant to cellular control mechanisms. In this case, vCYC expression may reestablish cell cycle homeostasis in the presence of active cellular antiviral responses that would otherwise induce cell cycle arrest.

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ADDENDUM

After submitting this article we learned of the publication by Dittmer et al. (12a) of transcriptional mapping results for the KSHV LT1 and LT2 transcripts which are similar to our own results reported here.

REFERENCES
