Leucine Zipper Domain Is Required for Kaposi Sarcoma-associated Herpesvirus (KSHV) K-bZIP Protein to Interact with Histone Deacetylase and Is Important for KSHV Replication*

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The Kaposi sarcoma-associated herpesvirus (KSHV; or human herpesvirus-8)-encoded protein called K-bZIP (also named K8) was found to be multifunctional. In this study, we discovered that K-bZIP interacts with histone deacetylase (HDAC) 1/2 in 12-O-tetradecanoylphorbol-13-acetate-stimulated BCBL-1 lymphocyte cells. K-bZIP appears to repress HDAC activity through this interaction, which we determined to be independent of K-bZIP SUMOylation. We dissected the domains of K-bZIP and found that the leucine zipper (LZ) domain is essential for the interaction of K-bZIP and HDAC. In addition, we constructed a KSHV bacterial artificial chromosome (BAC) with LZ domain-deleted K-bZIP (KSHVdLZ) and transfected this mutated KSHV BAC DNA into HEK 293T cells. As a result, it was consistently found that K-bZIP without its LZ domain failed to interact with HDAC2. We also showed that the interaction between K-bZIP and HDAC is necessary for the inhibition of the lytic gene promoters (ORF50 and OriLyt) of KSHV by K-bZIP. Furthermore, we found that the LZ domain is also important for the interaction of K-bZIP with the promoters of ORF50 and OriLyt. Most interestingly, although it was found to have suppressive effects on the promoters of ORF50 and OriLyt, KSHVdLZ replicates at a significantly lower level than its BAC-derived revertant (KSHVdLZRev) or KSHVWT (BAC36) in HEK 293T cells. The defectiveness of KSHVdLZ replication can be partially rescued by siRNA against HDAC2. Our results suggest that the function of K-bZIP interaction with HDAC is two-layered. 1) K-bZIP inhibits HDAC activity generally so that KSHVdLZ replicates at a lower level than does KSHVWT. 2) K-bZIP can recruit HDAC to the promoters of OriLyt and ORF50 through interaction with HDAC for K-bZIP to have a temporary repressive effect on the two promoters.

Kaposi sarcoma-associated herpesvirus (KSHV)2 is associated with Kaposi sarcoma (1, 2) and several B cell malignancies, such as primary effusion lymphoma and multicentric Castleman disease (1, 3–6). Cell types identified for successful KSHV infection include monocytes, endothelial/spindle cells, B cells, and epithelial cells (6–11). After primary infection, KSHV can set up a latent infection in the host cells where KSHV genomes exist as episomes in the nucleus, and the latent infection can be reactivated to a lytic infection to produce and release infectious viral particles (10, 12, 13). Accumulating studies have revealed that the KSHV latency to lytic switch is important in viral pathogenesis, in secondary infection (to maintain the number of infected cells), and in tumorogenesis (14–17). Unfortunately, the mechanism by which KSHV is reactivated from latent infection is still unclear.

The findings that histone deacetylase (HDAC) inhibitors, such as sodium butyrate, can reactivate KSHV from latent infection (18, 19) suggest that procedures affecting the activities of histone acetylase and HDAC might be related to viral reactivation. HDACs are a category of enzymes with the ability to change substrates from the acetylated to the deacetylated state, which results in a tighter chromatin structure (for histones) or reduced transactivation activity of other substrates, such as gene transcription regulators like p53 (19, 20). HDAC1 and -2 are the Class I proteins of the category and exist as abundant nuclear proteins in all kinds of mammalian cells (21). HDAC1 and -2 always exist together and are found in three different complexes (Sin3, NuRD/NRD/Mi2, and CoREST) (20). HDACs have been found to be associated with several herpesviruses, such as cytomegalovirus (CMV) and herpes simplex type 1 (HSV-1), interacting with viral promoters or viral proteins and negatively affecting their gene expression, leading to subsequent reduction in viral replication (22–25). Several

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2 The abbreviations used are: KSHV, Kaposi sarcoma-associated herpesvirus; HDAC, histone deacetylase; SUMO, small ubiquitin-like modifier; LZ, leucine zipper; BAC, bacterial artificial chromosome; RTA, replication and transcription activator; CBP, CAMP-response element-binding protein (CREB)-binding protein; PML, promyelocytic leukemia protein; TPA, 12-O-tetradecanoylphorbol-13-acetate; ChIPed, chromatin immunoprecipitated; EBV, Epstein-Barr virus; bZIP, basic leucine zipper.
viral proteins (e.g. IE1/2 of human CMV, IE1/3 of mouse CMV, and ICP0 of HSV-1) have been identified as interacting with HDAC to repress its effects in favor of viral replications (22, 24); in other words, these may be viral strategies to repress their cellular repressor HDAC. We wondered whether HDACs might also play a role in KSHV reactivation and whether K-bZIP could have a repressive effect on HDAC.

Two KSHV-encoded lytic proteins, K-bZIP (also called K8) and ORF50 (also called replication and transcription activator (RTA)), are both immediate early proteins that have been recently scrutinized regarding their effects on KSHV gene promoters (26–30). K-bZIP (encoded by the K8 gene) is an early KSHV protein whose expression depends on ORF50, and the K-bZIP gene overlaps with ORF50 and needs to be spliced (31–33). Studies showed that 34 KSHV promoters can be activated by ORF50, whereas K-bZIP was found to be able to activate 21 KSHV promoters. The ORF50-encoded protein is the first de novo synthesized protein following reactivation and is required for KSHV to switch to the lytic stage (34). OriLyt DNA is involved in viral DNA replication, and its sequence encodes a 1.4-kb polyadenylated RNA (35–37). The regulation of both the ORF50 and OriLyt promoters is important to KSHV replication. However, the effects of K-bZIP on these two important promoters remain unclear and need to be confirmed.

K-bZIP has a number of functional domains: a transcription activation domain at the N terminus (amino acids 1–121) (40), a SUMO interaction motif (amino acids 75–82) (41), a leucine zipper domain at its C terminus (4, 31, 33), a DNA binding domain (amino acids 121–189), and a basic region 1–121) (40), a SUMO interaction motif (amino acids 75–82) (41). K-bZIP can be SUMOylated at lysine 158 (43), and this SUMOylation affects its interaction with many cellular and viral proteins (19). Several cellular proteins are known to interact with K-bZIP, including p53, CAMP-response element-binding protein (CREB)-binding protein (CBP), CCAAT/enhancer-binding protein α, and others (36, 40, 44–48). The consequences of the interaction of K-bZIP with cellular proteins on gene regulation have been found to be either negative or positive for viral growth. K-bZIP was discovered to interact with ORF50 and repress ORF50 activities of transactivation (49), suggesting that K-bZIP has repressive effects on viral gene expression and viral replication. However, knockdown of K-bZIP either abolishes the reactivation of KSHV, which implies that K-bZIP is an essential gene for KSHV lytic infection (50), or lowers viral DNA copies at the latent stage of viral infection, which suggests a possible role of K-bZIP in abortive lytic DNA replication of de novo infection or in the maintenance of latent viral genomes (51).

Here, we found that K-bZIP interacts and colocalizes with HDAC1/2 in the DNA replication domain, implying that K-bZIP might function through either recruiting HDAC (to have a negative effect on some gene regulation) or by segregating HDAC and inhibiting its activity (to have a positive effect on gene regulation). In this study, we discovered that the leucine zipper domain, not the SUMOylation of K-bZIP, is required for K-bZIP to interact with HDAC1/2 and with some KSHV lytic gene promoters and that these interactions are important for KSHV to replicate in HEK 293T cells.

**MATERIALS AND METHODS**

**Cell Culture**—The following cell lines were used: BCBL-1, B lymphocytes with KSHV latency; Ad5 E1A-transformed human epithelial kidney cell (HEK 293, ATCC CRL-1573); and 293 with SV40 large T-antigen (HEK 293T, ATCC CRL-11268). BCBL-1 cells were maintained in RPMI 1640 medium with 10% fetal calf serum (FCS) and 1% penicillin-streptomycin in a 37 °C incubator with 5% CO2. HEK 293 and HEK 293T cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FCS and 1% penicillin-streptomycin at 37 °C with 5% CO2.

**Molecular Cloning and Site-directed Mutagenesis of KSHV**

**BAC**—To mutate the K-bZIP gene, we used overlapping PCR to produce a mutated DNA fragment (for a diagram, see Fig. 4B) that was then cloned into a full-length K-bZIP cDNA in pcDNA3 to replace wild type with mutant sequence. The mutations were verified by DNA sequencing. To generate KSHV BAC with LZ-deleted K-bZIP, we used a galK counterselection BAC system (52). Briefly, we first replaced K-bZIP gene with a galK cassette (generated by PCR using the primers in Table 1) based on BAC36 (53). The recombinant bacteria (SW102) can only grow on minimal medium with galactose as the only carbon source and yield red colonies. Then the galK cassette was replaced by a PCR product that contains LZ-deleted or BAC-derived revertant K-bZIP DNA (generated by PCR using the primers shown in Table 1). This is achieved by selecting against the galK cassette by resistance to 2-deoxygalactose on minimal plates with glycerol as the carbon source. 2-Deoxygalactose is harmless unless phosphorylated by functional galK. Phosphorylation by galK turns 2-deoxygalactose into 2-deoxygalactose 1-phosphate, a non-metabolizable and therefore toxic intermediate that killed non-transformants. The resultant KSHV BACs (BACdLZ and BACdLZRev) were further verified by DNA sequencing of K-bZIP gene.

**Luciferase Assay**—Cells were collected 24 h after co-transfection of K-bZIP- and/or HDAC2-expressing plasmids with a luciferase reporter gene directed by the promoter of ORF50 (genomic location: 70561–71598, Genbank™ accession number U75698 (54), pORF50-luc), ORF59 (genomic location: 96737–98034, accession number U75698, pORF59-luc), or OriLyt (genomic location: 24093–24342, accession number U75698, pOriLyt-luc). pRL-TK was included in each transfection system as an internal control for the normalization of the DNA transfection. The Dual-Luciferase reporter assay system (Promega, Turner Designs) was used to examine the responsiveness of the promoters to K-bZIP and/or HDAC2. Each assay was performed in triplicate, and the luciferase activity was normalized by the amounts of total protein. Transfection efficiency was normalized with the Renilla luciferase activities (pRL-TK). The cell lysates were assayed for firefly luciferase and Renilla luciferase activities by using a TD-20/20 luminometer with a dual autoinjector (Promega, Turner Designs). The luciferase assays were carried out according to the manufacturer’s instructions (Promega).
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**HDAC Activity Assay**—HDAC activity was assessed with the HDAC activity assay kit (Upstate-Millipore, Lake Placid, NY) according to the manufacturer’s instructions. Immune complexes were incubated with 20,000 cpm [3H]acetyl-labeled histone H4 peptide (Upstate-Millipore, Lake Placid, NY) in 1× HDAC buffer at room temperature for 24 h with rolling. Reactions were stopped by adding 50 μl of 0.12 N acetic acid, 0.72 N HCl. The released acetate was extracted in 0.5 ml of ethyl acetate and mixed in 5 ml of scintillation solution, and radioactivity was measured in a scintillation counter. All assays were performed in triplicate.

**Antibodies**—Mouse anti-HDAC1 (ab31263), rabbit anti-HDAC1 (ab19845), rabbit anti-HDAC2 (ab32117), and mouse anti-HDAC2 antibodies (3F3, ab51832) were purchased from Abcam Inc. (Cambridge, MA; 1:250 for immunofluorescence; 1:1000 for Western blot). Monoclonal antibody against tubulin (T-9026) was purchased from Sigma-Aldrich (1:1000 for Western blot). Polyclonal antibody against PML (sc-5621), monoclonal antibody against Ac-histone H3 (Lys-24) (sc-34262), and monoclonal anti-enhanced GFP (sc-9966) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA; 1:100 for immunofluorescence to show PML and 1:1000 for Western blot to probe enhanced GFP). Anti-Kb-ZIP (K8) was a gift from Dr. Yuan (University of Pennsylvania) (37).

**Nick Translation**—Double-stranded DNA probes for *in situ* hybridization and Southern blot assays were labeled by nick translation as described previously (55). Briefly, 1 μg of plasmid DNA (BAC36; the whole KSHV genome was cloned in the BAC vector, which was a gift from Dr. S. J. Gao) (53), 10× nick translation buffer, 0.05 mM dNTP (dATP, dCTP, and dGTP), 0.01 mM dTTP, 0.04 mM biotinylated UTP, 1 unit of DNA polymerase I, and appropriate concentrations of DNase I were incubated at 15 °C for 50 min. Labeled fragments obtained from the protocol were 200–500 bases long as determined on 2% agarose gels.

**Immunocytochemistry and Fluorescence in Situ Hybridization**—For the immunofluorescence assay of adherent cells, cells were grown on round coverslips (Corning Glass Inc., Corning, NY) in 24-well plates (Falcon, BD Biosciences). For immunofluorescence assays in BCBL-1 cells, cells were washed with PBS and scraped into fresh Eppendorf tubes. Cell pellets were resuspended in cold buffer A (10 mM HEPES-KOH, pH 7.9 at 4 °C, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride) and incubated at 4 °C for 10 min. After centrifugation, pellets were resuspended in cold buffer C (20 mM HEPES-KOH, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride) by vortexing and incubated at 4 °C for 30 min. Clarified extracts were transferred to fresh tubes and stored at −70 °C until use.

**Chromatin Immunoprecipitation (ChIP) Assay and Real Time PCR**—HEK 293T cells harboring BACdLZ, its BAC-derived revertant, or BAC36 were stimulated with TPA (20 ng/ml) and sodium butyrate (0.5 mM) for 24 h and fixed with 1% formaldehyde. A ChIP assay was performed using the EZChIP kit.
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QUESTIONS

1. What are the main findings of the study regarding the interaction of KSHV K-bZIP with host proteins?

2. How were the DNA replication domains determined in the study?

3. What methods were used to detect the presence of K-bZIP and host proteins in the study?

4. What is the significance of the colocalization of K-bZIP and host proteins in the viral replication process?

5. How does the study contribute to our understanding of KSHV latency and reactivation?

6. What are the potential implications of these findings for the development of antiviral therapies?
recruited to K-bZIP domains (Fig. 2, C and F) some time between 12 and 48 h. These results suggest that cellular defensive proteins (HDAC1 and HDAC2) respond to the reactivation process of KSHV and might play a critical role in blocking lytic gene expression.

K-bZIP Interacts with HDAC and Reduces HDAC Activity

To determine the manner in which HDAC1 and HDAC2 are recruited into the DNA replication domains, we asked whether K-bZIP interacts with HDAC1 or HDAC2. Nuclear extracts were prepared from BCBL-1 cells with or without TPA treatment and incubated with protein G beads conjugated with anti-K-bZIP (mouse), anti-HDAC1 (mouse), or anti-HDAC2 (mouse) antibody or normal IgG from mouse as a control. Pulled down proteins were then detected by Western blot assays using antibodies to detect the respective proteins as indicated on the right. To avoid the heavy chain of IgG, we used secondary antibodies from TrueBlot ULTRA (eBioscience; catalog number 18-8817 for mouse or 18-8816 for rabbit).

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FIGURE 2. Distribution of K-bZIP and HDAC at late time of TPA treatment of BCBL-1. A–J, BCBL-1 cells first were treated with TPA (20 ng/ml) for 48 h, then fixed and permeabilized, and finally stained with primary antibodies to detect proteins as indicated and then with Texas Red-labeled secondary antibody. The primary antibodies include anti-K-bZIP (A, D, G, and J), -HDAC1 (B), -HDAC2 (E), and -PML (H). The panels show K-bZIP alone (red; A, D, and G), HDAC1 alone (green; B), and HDAC2 alone (green; E). KSHV DNA was shown by FISH (green; H and J), and merging is shown in C, F, I, and J. Scale bar, 20 μm. K, Southern blot assay to examine the DNA replication in BCBL-1 cells after being treated or untreated with TPA. w/o, without.

FIGURE 3. A, interactions of K-bZIP with HDACs. Nuclear extracts were prepared from BCBL-1 cells (treated or untreated with TPA for 24 h). Mouse (m) anti-K-bZIP, -HDAC1, and -HDAC2, and mouse IgG (mIgG) were bound to protein G beads and incubated with nuclear extracts. The eluted protein complexes were detected by Western blot using antibodies to detect the respective proteins as indicated on the right. To avoid the heavy chain of IgG, we used secondary antibodies from TrueBlot ULTRA (eBioscience; catalog number 18-8817 for mouse or 18-8816 for rabbit). B, effect of K-bZIP on HDAC2 activity. BCBL-1 cells were untreated (lanes 1 and 3) or treated (lanes 2 and 4) with TPA for 48 h. A total of 1.2 mg of cell lysates was immunoprecipitated with anti-HDAC2 (left) or anti-K-bZIP (right) antibodies. One-third of the precipitant was assayed for deacetylase activity with or without 1 μM sodium butyrate. The results shown for deacetylation activity are the averages of three independent assays. IP, immunoprecipitation. The S.E. of three assays was shown as the error bar.
HDAC2 antibody (because HDAC1 and -2 exist in the same complexes, we only used anti-HDAC2 antibody) or anti-K-bZIP antibody and used for the assay. The results showed that HDAC2-associated deacetylation activity decreased slightly after treatment with TPA for 48 h (Fig. 3B). HDAC2 activity from the complex co-immunoprecipitated by anti-K-bZIP antibody was reduced significantly (Fig. 3B). However, the K-bZIP-associated HDAC activity was reduced by an HDAC inhibitor, sodium butyrate, suggesting that the K-bZIP-bound HDAC(s) may retain at least partial deacetylation activity that is sensitive to sodium butyrate.

Leucine Zipper Domain, Not K-bZIP SUMOylation, Is Important for Interaction of K-bZIP and HDAC2—To determine whether KSHV DNA replication is required for the interaction of K-bZIP and HDAC2, we transfected a K-bZIP-expressing plasmid into HEK 293 cells free of the other components of KSHV. Nuclear extracts were prepared 24 h post-transfection and incubated with anti-K-bZIP antibody (mouse) or anti-HDAC2 antibody (rabbit)–conjugated beads, and Western blot assays were performed to check proteins in the complexes (Fig. 4A). As shown, HDAC2 can be pulled down by anti-K-bZIP antibody; K-bZIP can also be pulled down by anti-HDAC2 antibody. Therefore, K-bZIP interaction with HDAC2 was not dependent on KSHV DNA replication.

SUMOylation can affect the location of a protein in the nucleus and its interaction with other molecules. K-bZIP can be SUMOylated, so we wondered whether SUMOylation affects the interaction of K-bZIP with HDAC2. The K-bZIP protein SUMOylation motif has been mapped to a single amino acid residue (lysine 158) (43), and a SUMO interaction motif has been identified in amino acids 72–75 (41). We constructed several deletion mutations and a point mutation of K-bZIP (Fig. 4B), including pK8_K158R point mutant, pK8_dl72–75 (deleted SUMO interaction motif), pK8_dl122–132 (deleted nuclear localization signal), and pK8_dl205–219 (deleted leucine zipper domain). After co-transfection of these plasmids with pgfpSUMO-1 (SUMO-1 was N-terminally fused with GFP) into HEK 293 cells, the cell lysates were run in a Western blot assay using anti-GFP and anti-K-bZIP antibodies as indicated in Fig. 4B (right). As can be seen, K-bZIPs with mutated

FIGURE 4. Interactions of K-bZIP with HDAC2. A, nuclear extracts were prepared from HEK 293 cells that were transfected with wild-type (WT) pK-bZIP. Mouse (m) anti-K-bZIP, mouse IgG, rabbit (r) anti-HDAC2, and rabbit IgG were bound to protein G beads and incubated with nuclear extracts. The eluted protein complexes were detected by Western blot using antibodies to check the respective proteins as indicated on the right. B, HEK 293 cells were co-transfected with pgfpSUMO-1 and K-bZIP-expressing plasmids (mutation diagram is shown on the left) for 24 h. Cell lysates were assayed by Western blot using antibodies as indicated on the right. C, nuclear extracts were prepared from HEK 293 cells that were transfected with wild type or mutated pK-bZIP. Mouse anti-K-bZIP, mouse IgG (mlG), rabbit anti-HDAC2, and rabbit IgG (rlG) were bound to protein G beads and incubated with nuclear extracts. The eluted protein complexes were detected by Western blot using antibodies to detect the respective proteins as indicated on the right. D, immunofluorescence assay to show K-bZIP with deleted LZ domain distribution in the nucleus. IP, immunoprecipitation.
FIGURE 5. Interaction of K-bZIP and HDAC2 with KSHV gene promoters and transcription regulation of OriLyt, ORF50, and ORF59 promoters by K-bZIP, ORF50, HDAC, and K-bZIP mutants. A, HEK 293 cells were co-transfected with reporter plasmids containing the firefly luciferase gene under the control of the OriLyt (lower), ORF59 (middle), or ORF50 (upper) promoter with pcDNA3 (to normalize the input DNA amount), K-bZIP-, or ORF50-expressing plasmid. Renilla luciferase plasmid was included in each transfection as an internal control. At 24 h post-transfection, Dual-Luciferase assays were performed with the cell lysates of transfected cells. Relative luciferase activities were calculated by dividing the normalized firefly luciferase activity of each reporter by that of the pGL3 plasmid in pcDNA3-transfected cells. B, ChIP assay. HEK 293T cells harboring BAC36, BACdLZ, or its BAC-derived revertant were fixed with 1% paraformaldehyde at 24 h post-treatment with TPA (20 ng/ml) and sodium butyrate (0.5 mM). The whole cell lysates were sonicated to fragment the DNA. The DNA was then ChIPed using anti-HDAC2, anti-K-bZIP, or anti-Ac-histone H3 (AcH3) antibody. The ChIP assay was performed using an EZChIP kit (Upstate-Millipore, Billerica, MA) according to the manufacturer’s protocol. The ChIPed DNAs were finally examined by real time PCR using the primers amplying promoters of ORF50, ORF59, and OriLyt (Table 1). The relative DNA quantities were calculated by comparing the PCR signals from normal IgG and specific antibodies with that from input. C, the same as in A but different plasmids (K-bZIP mutants) were applied for co-transfection. Results in A and C are mean ± S.D. from three independent assays.
SUMOylation target (K158R), deleted SUMO interaction motif, and deleted nuclear localization signal were not SUMOylated. However, K-bZIP with loss of SUMOylation can still be pulled down by anti-HDAC2 antibody in a co-immunoprecipitation assay (Fig. 4C, left). Therefore, SUMOylation of K-bZIP is not important for its interaction with HDAC1/2.

To map out the domain that is essential for the interaction of K-bZIP with HDAC1/2, we deleted the leucine zipper domain, pK8_dL205–219. K-bZIP with a deleted leucine zipper domain can still be SUMOylated (Fig. 4B, right) but loses the ability to interact with HDAC2 in the co-immunoprecipitation assay (as shown in Fig. 4C, right). Therefore, the leucine zipper domain is essential for the interaction of K-bZIP with HDAC2. We found that the distribution pattern of K-bZIP with deleted LZ also changed, losing the punctate nuclear pattern and becoming diffuse (Fig. 4D).

K-bZIP Protein Represses Several KSHV Lytic Promoters via Its Interaction with HDAC2—A comprehensive test of KSHV gene activation by ORF50 and K-bZIP proteins was conducted recently (27). However, the effects of K-bZIP on important lytic stage promoters (including ORF50, ORF59, and OriLyt) were not clear. Previous unpublished experiments performed in our laboratory have repeatedly revealed that K-bZIP has inhibitory effects on the promoters of ORF50 and OriLyt, effects that were not evident in the global detection assays (27). For that reason, we performed co-transfection of the luciferase reporter plasmids with ORF50 or OriLyt with the luciferase-tagged reporter plasmids with ORF50 or OriLyt with K-bZIP. However, pK-bZIP158KR lost the ability to repress ORF50 and OriLyt nearly as well as wild-type K-bZIP. That the effect is prominent only in ORF50 and OriLyt suggests that the K-bZIP leucine zipper domain must be important for K-bZIP to recruit HDAC2 to the promoters of ORF50 and OriLyt.

Finally, we wanted to know whether the interaction of K-bZIP with HDAC2 is important for the repression of the two promoters by K-bZIP. We co-transfected the HEK 293 cells with the luciferase-tagged reporter plasmids with ORF50 or OriLyt with K-bZIP. The repressive function of K-bZIP might be due to recruitment of HDAC2 to the promoters of K-bZIP.

### Table 1

| Purpose of the primer | DNA sequence |
|-----------------------|--------------|
| K-bZIP gene | 5′-ATGCCCAAATGAAGGAC-3′  
Reverse | 5′-TCAACATGGGGAGTGG-3′ |
| OriLyt promoter | 5′-CTTACATTGACCCTGTCC-3′  
Reverse | 5′-TGTCGCCCCGGTCCTCGT-3′ |
| ORF50 promoter | 5′-ATGAAGATGTGGTACAGCA-3′  
Reverse | 5′-TAGGGCCATCTCGGCC-3′ |
| ORF59 promoter | 5′-CACAACCCACACCTGGGCT-3′  
Reverse | 5′-CGGACAGGAAAATCAGAG-3′ |
| BAC mutagenesis | Homo-galK  
Forward | 5′-CTGCTGCCGGGGCTCCTCGTT-3′  
Reverse | 5′-CCTACATGGGCAGCTTGTCC-3′ |
| Homo-K-bZIP | 5′-ATGCCCAAATGAAGGAC-3′  
Reverse | 5′-AACCAAGCTTCAACATGG-3′ |
| ORF73 gene | 5′-CGCGAATACCGCTATGCTCA-3′  
Reverse | 5′-GGAACGCCGTCCTACAGA-3′ |

**TABLE 1**

Primer DNA sequence

| Purpose of the primer | DNA sequence |
|-----------------------|--------------|
| K-bZIP gene | 5′-ATGCCCAAATGAAGGAC-3′  
Reverse | 5′-TCAACATGGGGAGTGG-3′ |
| OriLyt promoter | 5′-CTTACATTGACCCTGTCC-3′  
Reverse | 5′-TGTCGCCCCGGTCCTCGT-3′ |
| ORF50 promoter | 5′-ATGAAGATGTGGTACAGCA-3′  
Reverse | 5′-TAGGGCCATCTCGGCC-3′ |
| ORF59 promoter | 5′-CACAACCCACACCTGGGCT-3′  
Reverse | 5′-CGGACAGGAAAATCAGAG-3′ |
| BAC mutagenesis | Homo-galK  
Forward | 5′-CTGCTGCCGGGGCTCCTCGTT-3′  
Reverse | 5′-CCTACATGGGCAGCTTGTCC-3′ |
| Homo-K-bZIP | 5′-ATGCCCAAATGAAGGAC-3′  
Reverse | 5′-AACCAAGCTTCAACATGG-3′ |
| ORF73 gene | 5′-CGCGAATACCGCTATGCTCA-3′  
Reverse | 5′-GGAACGCCGTCCTACAGA-3′ |

Primer DNA sequence

**Interaction of KSHV K-bZIP Gene Product and Host Cells**

To know whether the K-bZIP or LZ-deleted K-bZIP could bind to the OriLyt or ORF50 promoters in HEK 293T cell lines, a ChIP assay using anti-HDAC2, anti-K-bZIP, or anti-acetylated histone (Ac-histone H3) antibody was performed. The ChIPPed DNAs were then examined by real time PCR using the primers amplifying promoters of ORF50, ORF59, and OriLyt (Table 1). As shown in Fig. 5B, after comparing the PCR signals from normal IgG and specific antibodies with that from input, we found that both HDAC2 and K-bZIP bind to promoters of ORF50 and OriLyt as does Ac-histone H3 in HEK 293T cells harboring BAC36 and BACdLZRev (Fig. 5B, upper and lower panels). Association of K-bZIP with the promoter of ORF50 was not obvious. Although the association of HDAC2 and Ac-histone H3 with all promoters is evident and was detected in HEK 293T cells harboring BACdLZ, the level of DNA ChIPed by anti-HDAC2 is significantly lower compared with that from cells with BAC36 and BACdLZRev (Fig. 5B). That the effect is prominent only in ORF50 and OriLyt suggests that the K-bZIP leucine zipper domain must be important for K-bZIP to recruit HDAC2 to the promoters of ORF50 and OriLyt.
structed a KSHV BAC DNA with LZ-deleted K-bZIP gene and its BAC-derived revertant (BACdLZRev). The BAC DNAs were verified as follows. 1) XhoI digestion showed that the pattern of KSHV BACdLZ was indistinguishable from that of its BAC-derived revertant or BAC36 (Fig. 6A). 2) PCR of K-bZIP gene showed a slightly smaller band from KSHVdLZ than that from its BAC-derived revertant or BAC36 (Fig. 6B). 3) DNA sequencing showed that amino acids 205–219 were deleted in-frame (data not shown).

We then transfected the BAC DNA (BAC36, BACdLZ, or BACdLZRev) into HEK 293T cells to make HEK 293T cell lines harboring KSHV genomes: HEK 293T/BAC36, 293T/BACdLZ, and 293T/BACdLZRev. The transiently transfected cells were purified by cell sorting of a GFP marker so that nearly all cells were KSHV BAC-positive. After treatment with TPA for different times as indicated in Fig. 6C, we collected the whole cell lysate samples. KSHV proteins (K-bZIP, RTA, latency-associated nuclear antigen, and ORF45) were then analyzed by Western blotting assay using antibodies against latency-associated nuclear antigen (LANA), RTA, K-bZIP, and ORF45. Tubulin was used for controlling the sample loading. D, co-immunoprecipitation assays were performed to determine the interaction of K-bZIP with HDAC2 using the nuclear extracts from HEK 293T cells harboring BAC36, BACdLZ, and BACdLZRev. Results show that K-bZIP with deleted LZ fails to interact with HDAC2 (middle panel), whereas WT K-bZIP interacts with HDAC2 (left and right panels).

We wished to determine whether the LZ domain of K-bZIP and HDAC2 are important for KSHV to replicate in HEK 293T cells. Cells harboring BAC36, BACdLZ, or BACdLZRev were prepared in 6-well plates and treated with TPA for different times as indicated in Fig. 7 (upper panel). Cells were collected together with medium and treated with protease K, and total viral DNA was extracted. KSHV DNA from cells was quantified by real time PCR compared with an external BAC36 standard. As can be seen, KSHV-BACdLZ replicated at a lower level than its BAC-derived revertant and KSHV-BAC36, especially at the late time point of 48 h. This might suggest that the LZ is important for late (48 h) but not early (24 h or less) actions of K-bZIP. Taken together, the leucine zipper domain is important for…
K-bZIP to interact with some KSHV promoters and for KSHV replication in HEK 293T cells. The interaction of K-bZIP with HDAC has two different functions: 1) to repress HDAC activity as shown in Fig. 3B to favor viral replication and 2) to recruit HDAC to and repress promoters of ORF50 and OriLyt. However, the general effects of K-bZIP through interaction with HDAC are apparently positive for KSHV replication because abolishing interaction with HDAC by deleting the LZ domain produced a defective phenotype of viral gene expression and DNA replication.

To further demonstrate that the effects of K-bZIP on KSHV DNA replication are connected to HDAC activity, we knocked down HDAC2 using small hairpin RNA carried by a lentivirus against HDAC2 (shRNA plasmid: sc-44262-SH) from HEK 293T cells harboring the three different BAC DNAs. The effect of the siRNA to specifically inhibit HDAC2 gene expression is shown by Western blot assay in Fig. 7, right, lower panel. Interestingly, the defectiveness of KSHVdLZ replication can be significantly rescued by the inhibition of HDAC2 gene expression as shown in Fig. 7, left, lower panel. Taken together, although the K-bZIP has a negative effect on some KSHV promoters, its general function in KSHV is enhancing viral DNA replication through inhibiting HDAC activity.

DISCUSSION

Interest in K-bZIP of KSHV originated from the fact that it is the positional homologue of Zta, which is a reactivator in EBV (6, 34, 39, 54, 62). Both Zta of EBV and K-bZIP of KSHV are related to the basic leucine zipper (bZIP) family of transcription factors; moreover, both genes are adjacent to another conserved transcription activator, RTA. However, after comparison of the functions and protein structures of K-bZIP of KSHV with Zta of EBV, it was recognized that the two proteins have very limited similarities (33). First, KSHV K-bZIP alone cannot switch KSHV from latent to lytic infection (33). Second, within the amino acid sequence, K-bZIP of KSHV and EBV Zta are not significantly homologous (33). Finally, K-bZIP of KSHV lacks a basic DNA binding region adjacent to its dimerization domain, and it has not been demonstrated to interact with DNA directly (33). Therefore, KSHV K-bZIP might have different functions than does EBV Zta.

The role of K-bZIP in gene regulation has been widely investigated, and it is believed to be essential for KSHV reactivation. However, the part it plays in KSHV reactivation is not yet fully understood. Accumulated evidence shows that K-bZIP can interact with different cellular and viral proteins to present both trans-repressive and trans-activating activities (29, 40, 44, 63, 64). K-bZIP can interact with SUMO (43), ND10 components (60, 65), CBP (44, 66), and CCAAT/enhancer-binding protein (67). Although it has several functions, its primary function is to arrest cell cycle progression in the G1 phase (65, 68); this is accomplished by its interaction with cellular proteins and results in the regulation of cell cycle protein production (65, 67). Its post-translational modifications (including SUMOylation) have also attracted tentative interest because they are related to the repressive effect of K-bZIP on gene regulation (43) and because in addition SUMOylation often aids protein interaction (69). K-bZIP protein was discovered to inhibit TGF-β1 signaling through interaction with CBP (44). CBP is an acetylase and modifies histone structure to loosen DNA conformation, making it more accessible for gene transcription factors.

HDAC family proteins deacetylate histones and have the opposite function of CBP. Using a co-immunoprecipitation assay, we observed that K-bZIP can interact with HDAC1 and
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-2. And yet, both HDAC1 and HDAC2 are present in the DNA replication domain, and both colocalize with K-bZIP; for this reason, we believe that K-bZIP has additional, as yet unverified functions. The interactions of K-bZIP and HDAC were not dependent on KSHV DNA replication because K-bZIP interacts with HDAC in a co-transfection system. SUMO modification did not affect the interaction of K-bZIP with HDAC. Moreover, LZ-deleted K-bZIP failed to interact with HDAC, demonstrating that the leucine zipper domain is essential for the interaction.

However, the functions of K-bZIP on KSHV promoters are not the same. On the one hand, in a co-transfection system using a luciferase assay, we found that the interaction of K-bZIP with HDAC is required for K-bZIP to perform its inhibitory function on KSHV promoters ORF50 and OriLyt. On the other hand, K-bZIP interaction with HDAC can directly repress the activity of HDAC, and a recent comprehensive study of the function of K-bZIP showed that it can activate 21 other KSHV promoters (16). These data suggest that the repression by K-bZIP of some promoters may be important for the maintenance of latency, whereas later activation of other promoters will be part of the lytic reactivation pathway. In the established HEK 293T cells harboring KSHV BAC, after treatment with TPA, KSHV with a LZ-deleted K-bZIP had a reduced replication phenotype. This implies that the repressive effect of K-bZIP on HDAC activity is more important than its effect on some viral promoters, explaining why KSHV-dLZ has a defective phenotype.

Several herpesviral proteins have been discovered to interact with HDAC and to have different functions. CMV IE1 interacts with HDAC, represses deacetylase activity, and enhances viral replication (22, 56). However, IE2 of CMV interacts with HDAC2 and has repressive effects on several promoters (70). Here, we are the first to report that KSHV K-bZIP interacts with HDAC, playing an important role in enhancing viral DNA replication. Its repressive effects on some important KSHV promoters (such as ORF50 and OriLyt) seem to contradict its importance in KSHV replication. We were curious whether the repressive effects of K-bZIP on promoters require interaction with HDAC because HDAC is generally an inhibitor of gene expression. ChIP assay results (Fig. 5B) suggested that 1) HDAC2 can bind to the promoter of ORF59 that is not associated with K-bZIP, 2) the leucine zipper domain is important for the interaction of K-bZIP with the promoters of ORF50 and OriLyt that might be mediated by HDAC, and 3) the repressive effects of K-bZIP on KSHV promoters might work through a direct interaction with HDAC and promoters. The repressive effects of OriLyt and ORF50 on KSHV promoters did not result in lower KSHV replication (Fig. 7). The repressive effects of K-bZIP on HDAC activity are important to KSHV replication because the knockdown of HDAC2 partially complements KSHV-dLZ replication in HEK 293T cells.

In summary, we discovered that K-bZIP interacts with HDAC1/2; this interaction might be crucial for presenting HDAC1/2 in viral DNA replication domains. We found that the leucine zipper domain is essential for the interaction of K-bZIP and HDAC2 and that this interaction is independent of SUMOylation. We also provide evidence that K-bZIP is able to repress HDAC deacetylase activity and interact with and inhibit the lytic gene promoters (ORF50 and OriLyt) of KSHV. Our results suggest that K-bZIP might regulate KSHV gene expression through interacting with HDAC. Most importantly, our observations that the leucine zipper domain of K-bZIP is important for KSHV replication in HEK 293T cells and that K-bZIP can inhibit HDAC activity suggest that this inhibition of HDAC plays an important role in viral replication. Our model for the function of K-bZIP interaction with HDAC has two layers. 1) K-bZIP can bring HDAC molecules to some promoters, thereby having a repressive effect, and 2) it can bind to HDAC and inhibit the effects of the deacetylase, which in turn causes K-bZIP to have a positive effect on KSHV replication.

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REFERENCES

1. Soulier, J., Grollet, L., Oksenhendler, E., Cacoub, P., Cazals-Hatem, D., Babinet, P., d’Agay, M. F., Clauvel, J. P., Raphael, M., Degos, L., and Sigaux, F. (1995) Kaposi’s sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman’s disease. Blood 86, 1276–1280
2. Chang, Y., Cesaran, E., Pissis, M. S., Lee, F., Culeppe, J., Knowles, D. M., and Moore, P. S. (1994) Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi’s sarcoma. Science 266, 1865–1869
3. Ambrozak, J. A., Blackbourn, D. J., Herndier, B. G., Glogau, R. G., Gullett, J. H., McDonal, A. R., Lennette, E. T., and Levy, J. A. (1995) Herpes-like viruses in HIV-infected and uninfected Kaposi’s sarcoma patients. Science 268, 582–583
4. Al Mejari, S., Cresolli, E. and Sinclair, A. J. (2005) Investigation of the multimerization region of the Kaposi’s sarcoma-associated herpesvirus (human herpesvirus 8) protein K-bZIP: the proposed leucine zipper region encodes a multimerization domain with an unusual structure. J. Virol. 79, 7905–7910
5. Cesaran, E., Chang, Y., Moore, P. S., Said, J. W., and Knowles, D. M. (1995) Kaposi’s sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N. Engl. J. Med. 332, 1186–1191
6. Dupin, N., Fisher, C., Kellam, P., Ariad, S., Tulliez, M., Franck, N., van Marck, E., Salmon, D., Gorin, I., Escande, J. P., Weiss, R. A., Altalato, K., and Boshoff, C. (1999) 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
7. Blasig, C., Zietz, C., Haar, B., Neipel, F., Esser, S., Brockmeyer, N. H., Tscharcier, E., Colombini, S., Ensoli, B., and Stürzl, M. (1997) Monocytes in Kaposi’s sarcoma lesions are productively infected by human herpesvirus-8. J. Virol. 71, 7963–7968
8. Boshoff, C., Schulz, T. F., Kennedy, M. M., Graham, A. K., Fisher, C., Thomas, A., McGee, J. O., Weiss, R. A., and O’Leary, J. J. (1995) Kaposi’s sarcoma-associated herpesvirus infects endothelial and spindle cells. Nat. Med. 1, 1274–1278
9. Diamond, C., Brodie, S. J., Krieger, J. N., Huang, M. L., Koelle, D. M., Diem, K., Muthui, D., and Corey, L. (1998) Human herpes virus 8 in the prostate glands of men with Kaposi’s sarcoma. J. Virol. 72, 6223–6227
10. Staskus, K. A., Zhong, W., Gebhard, K., Herndier, B., Wang, H., Renne, R., Beneke, J., Budney, I., Anderson, D. J., Ganem, D., and Haase, A. T. (1997) Kaposi’s sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells. J. Virol. 71, 715–719
11. Stürzl, M., Blasig, C., Schreier, A., Neipel, F., Hohenadl, C., Corneli, E., Ascherl, G., Esser, S., Brockmeyer, N. H., Ekman, M., Kaaya, E. E.
Interaction of KSHV K-bZIP Gene Product and Host Cells

Tschachler, E., and Biberfeld, P. (1997) Expression of HHV-8 latency-associated T0.7 RNA in spindle cells and endothelial cells of AIDS-associated, classical and African Kaposi’s sarcoma. Int. J. Cancer 72, 68–71

Ye, J., Gradoville, L., Daigle, D., and Miller, G. (2007) De novo protein synthesis is required for lytic cycle reactivation of Epstein-Barr virus, but not Kaposi’s sarcoma-associated herpesvirus, in response to histone deacetylase inhibitors and protein kinase C agonists. J. Virol. 81, 9279–9291

Miller, G., Heston, L., Grogan, E., Gradoville, L., Rigby, M., Sun, R., Shedd, D., Kushnaryov, V. M., Grossberg, S., and Chang, Y. (1997) Selective switch between latency and lytic replication of Kaposi’s sarcoma herpesvirus and Epstein-Barr virus in dually infected body cavity lymphoma cells. J. Virol. 71, 314–324

Carroll, K. D., Bu, W., Palermo, D., Spadavecchia, S., Lynch, S. J., Marras, S. A., Tyagi, S., and Lukac, D. M. (2006) Kaposi’s sarcoma-associated herpesvirus lytic switch protein stimulates DNA binding of RBP-Jk/CSL to activate the Notch pathway. J. Virol. 80, 9697–9709

Chang, P. J., Chen, L. W., Shih, Y. C., Tsai, P. H., Liu, A. C., Hung, C. H., Liu, J. Y., and Wang, S. S. (2011) Role of the cellular transcription factor YY1 in the latent-lytic switch of Kaposi’s sarcoma-associated herpesvirus. Virology 413, 194–204

Biberfeld, P., Ensoli, B., Stürzl, M., and Schulz, T. F. (1998) Kaposi sarcoma-associated herpesvirus/herpesvirus 8, cytokines, growth factors and HIV in pathogenesis of Kaposi’s sarcoma. Curr. Opin. Infect. Dis. 11, 97–105

Viejo-Borbolla, A., and Schulz, T. F. (2003) Kaposi’s sarcoma-associated herpesvirus (KSHV/HHV8): key aspects of epidemiology and pathogenesis. AIDS Rev. 5, 222–229

Lu, F., Day, L., Gao, S. J., and Lieberman, P. M. (2006) Acetylation of the latency-associated nuclear antigen regulates repression of Kaposi’s sarcoma-associated herpesvirus lytic transcription. J. Virol. 80, 5273–5282

Minucci, S., and Pelicci, P. G. (2006) Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat. Rev. Cancer 6, 52–61

Ng, H. H., and Bird, A. (2000) Histone deacetylases: silencers for hire. Curr. Opin. Gen. 9, 97–105

Kalejta, R. F. (2008) Functions of human cytomegalovirus tegument proteins prior to immediate early gene expression. J. Virol. 82, 10950–10960

Kato-Noah, T., Xu, Y., Rossetto, C. C., Colletti, K., Papoušková, I., and Pari, G. S. (2007) Overexpression of the Kaposi’s sarcoma-associated herpesvirus transactivator K-Rta can complement a K-bZIP deletion BACmid and yields an enhanced growth phenotype. J. Virol. 81, 13519–13532

Lin, S. F., Robinson, D. R., Miller, G., and Kung, H. J. (1999) Kaposi’s sarcoma-associated herpesvirus encodes a BZIP protein with homology to BZLF1 of Epstein-Barr virus. J. Virol. 73, 1909–1917

Tang, S., and Zheng, Z. M. (2002) Kaposi’s sarcoma-associated herpesvirus K8 exon 3 contains three 5′-splice sites and harbors a K8.1 transcription start site. J. Biol. Chem. 277, 14547–14556

Sinclair, A. J. (2003) BZIP proteins of human gammaherpesviruses. J. Gen. Virol. 84, 1941–1949

Seaman, W. T., Ye, D., Wang, R. X., Hale, E. E., Weisse, M., and Quinlin, E. B. (1999) Gene expression from the ORF50/K8 region of Kaposi’s sarcoma-associated herpesvirus. Virology 263, 436–449

Lin, C. L., Li, H., Wang, Y., Zhu, F. X., Kudchodkar, S., and Yuan, Y. (2003) Kaposi’s sarcoma-associated herpesvirus lytic origin (ori-Lyt)-dependent DNA replication: identification of the ori-Lyt and association of K8 bZIP protein with the origin. J. Virol. 77, 5578–5588

Wu, F. Y., Abn, I. H., Alcendor, D. J., Jang, W. J., Xiao, J., Hayward, S. D., and Hayward, G. S. (2001) Origin-independent assembly of Kaposi’s sarcoma-associated herpesvirus DNA replication components in transient cotransfection assays and association with the ORF-K8 protein and cellular PML. J. Virol. 75, 1487–1506

Yang, W., Chong, O. T., and Yuan, Y. (2004) Differential regulation of K8 gene expression in immediate-early and delayed-early stages of Kaposi’s sarcoma-associated herpesvirus. Virology 325, 149–163

Seaman, W. T., and Quinlin, E. B. (2003) Lytic switch protein (ORF50) response element in the Kaposi’s sarcoma-associated herpesvirus K8 promoter is located within but does not require a palindromic structure. Virology 310, 72–84

Gruiffat, H., Portes-Sentis, S., Sergeant, A., and Manet, E. (1999) Kaposi’s sarcoma-associated herpesvirus (human herpesvirus-8) encodes a homologue of the Epstein-Barr virus bZIP protein EB1. J. Gen. Virol. 80, 557–561

Park, J., Seo, T., Hwang, S., Lee, D., Gwack, Y., and Choe, J. (2000) The K-bZIP protein from Kaposi’s sarcoma-associated herpesvirus interacts with p53 and represses its transcriptional activity. J. Virol. 74, 11977–11982

Chang, P. C., Izumiya, Y., Wu, C. Y., Fitzgerald, L. D., Campbell, M., Ellison, T. J., Lam, K. S., Luciw, P. A., and Kung, H. J. (2010) Kaposi’s sarcoma-associated herpesvirus (KSHV) encodes a SUMO E3 ligase that is SIM-dependent and SUMO-2/3-specific. J. Biol. Chem. 285, 5266–5273

Portes-Sentis, S., Manet, E., Gourou, G., Sergeant, A., and Gruiffat, H. (2001) Identification of a short amino acid sequence essential for efficient nuclear targeting of the Kaposi’s sarcoma-associated herpesvirus/human herpesvirus-8 K8 protein. J. Gen. Virol. 82, 507–512

Izumiya, Y., Ellison, T. J., Yeh, E. T., Jung, J. U., Luciw, P. A., and Kung, H. J. (2005) Kaposi’s sarcoma-associated herpesvirus K-bZIP represses gene transcription via SUMO modification. J. Virol. 79, 9912–9925

Hwang, S., Gwack, Y., Byun, H., Lin, S. F., and Choe, J. (2001) The Kaposi’s sarcoma-associated herpesvirus K8 protein interacts with CREB-binding protein (CBP) and represses CBP-mediated transcription. J. Virol. 75, 9509–9516

Reinke, A. W., Grigoryan, G., and Keating, A. E. (2010) Identification of bZIP interaction partners of viral proteins HBZ, MEQ, BZLF1, and K-bZIP using coiled-coil arrays. Biochemistry 49, 1985–1997

Lefort, S., Soucy-Faulkner, A., Grandvaux, N., and Flamand, L. (2007) Binding of Kaposi’s sarcoma-associated herpesvirus K-bZIP to interferon-responsive factor 3 elements modulates antiviral gene expression. J. Virol. 81, 10950–10960

Tomita, M., Choe, J., Tsukazaki, T., and Mori, N. (2004) The Kaposi’s sarcoma-associated herpesvirus K-bZIP protein represses transforming growth factor β signaling through interaction with CREB-binding protein. Oncogene 23, 8272–8281

Izumiya, Y., Lin, S. F., Ellison, T. J., Levy, A. M., Mayeur, G. L., Izumiya, C., and Kung, H. J. (2003) Cell cycle regulation by Kaposi’s sarcoma-associated herpesvirus K-bZIP: direct interaction with cyclin-CDK2 and induc-
Interaction of KSHV K-bZIP Gene Product and Host Cells

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49. Liao, W., Tang, Y., Lin, S. F., Kung, H. J., and Giam, C. Z. (2003) K-bZIP of Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 (KSHV/HHV-8) binds KSHV/HHV-8 Rta and represses Rta-mediated transactivation. J. Virol. 77, 3809–3815

50. Lefort, S., and Flamand, L. (2009) Kaposi's sarcoma-associated herpesvirus K-bZIP protein is necessary for lytic viral gene expression, DNA replication, and virion production in primary effusion lymphoma cell lines. J. Virol. 83, 5869–5880

51. Wang, Y., Sathish, N., Hollow, C., and Yuan, Y. (2011) Functional characterization of Kaposi's sarcoma-associated herpesvirus open reading frame K8 by bacterial artificial chromosome-based mutagenesis. J. Virol. 85, 1943–1957

52. Warden, C., Tang, Q., and Zhu, H. (2011) Herpesvirus BACs: past, present, and future. J. Biomed. Biotechnol. 2011, 124595

53. Zhou, F. C., Zhang, Y. J., Deng, J. H., Wang, X. P., Pan, H. Y., Hettler, E., and Gao, S. J. (2002) Efficient infection by a recombinant Kaposi's sarcoma-associated herpesvirus cloned in a bacterial artificial chromosome: application for genetic analysis. J. Virol. 76, 6185–6196

54. Russo, J. J., Chien, M. C., Chen, J., Yan, M., Maddalena, D., Parry, J. P., Peruzzi, D., Edelman, I. S., Chang, Y., and Moore, P. S. (1996) Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). Proc. Natl. Acad. Sci. U.S.A. 93, 14862–14867

55. Wang, Y., Li, H., Tang, Q., Maul, G. G., and Yuan, Y. (2008) Kaposi's sarcoma-associated herpesvirus orf-Lyt-dependent DNA replication: involvement of host cellular factors. J. Virol. 82, 2867–2882

56. Bosshoff, C., and Chang, Y. (2001) Kaposi's sarcoma-associated herpesvirus: a new DNA tumor virus. Annu. Rev. Med. 52, 453–470

57. Wang, S. E., Wu, F. Y., Chen, H., Shamay, M., Zheng, Q., and Hayward, G. S. (2004) Early activation of the Kaposi's sarcoma-associated herpesvirus RTA, RAP, and MTA promoters by the tetradeacanoyl phorbol acetate-induced AP1 pathway. J. Virol. 78, 4248–4267

58. Wu, F. Y., Tang, Q. Q., Fujimuro, M., Chiou, C. J., Zheng, Q., Chen, H., Hayward, S. D., Lane, M. D., and Hayward, G. S. (2003) Cell cycle arrest by Kaposi's sarcoma-associated herpesvirus replication-associated protein is mediated at both the transcriptional and posttranslational levels by binding to CCAAT/enhancer-binding protein α and p21(CIP-1). J. Virol. 77, 8893–8914

59. Duan, L. M., Chao, M. F., Chen, M. Y., Shih, H. M., Chiang, Y. P., Huang, C. Y., and Lee, C. Y. (2001) Reciprocal regulatory interaction between human herpesvirus 8 and human immunodeficiency virus type 1. J. Biol. Chem. 276, 13427–13432

60. Wang, Y., Li, H., Tang, Q., Maul, G. G., and Yuan, Y. (2008) Kaposi's sarcoma-associated herpesvirus K8 protein interacts with hSNF5. J. Gen. Virol. 84, 665–676

61. Wu, F. Y., Tang, Q. Q., Fujimuro, M., Chiou, C. J., Zheng, Q., Chen, H., Hayward, S. D., Lane, M. D., and Hayward, G. S. (2003) Cell cycle arrest by Kaposi's sarcoma-associated herpesvirus replication-associated protein is mediated at both the transcriptional and posttranslational levels by binding to CCAAT/enhancer-binding protein α and p21(CIP-1). J. Virol. 77, 8893–8914

62. Wu, F. Y., Tang, Q. Q., Chen, H., ApRhys, C., Farrell, C., Chen, J., Fujimuro, M., Lane, M. D., and Hayward, G. S. (2002) Lytic replication-associated protein (RAP) encoded by Kaposi's sarcoma-associated herpesvirus causes p21CIP-1-mediated G1 cell cycle arrest through CCAAT/enhancer-binding protein-α. Proc. Natl. Acad. Sci. U.S.A. 99, 10683–10688

63. Yamanegi, K., Tang, S., and Zheng, Z. M. (2005) Kaposi's sarcoma-associated herpesvirus K8β is derived from a spliced intermediate of K8 pre-mRNA and antagonizes K8α (K-bZIP) to induce p21 and p53 and blocks K8α-CDK2 interaction. J. Virol. 79, 14207–14221

64. Johnson, E. S. (2004) Protein modification by SUMO. Annu. Rev. Biochem. 73, 355–382

65. Park, J. J., Kim, Y. E., Pham, H. T., Kim, E. T., Chong, Y. H., and Ahn, J. H. (2007) Functional interaction of the human cytomegalovirus IE2 protein with histone deacetylase 2 in infected human fibroblasts. J. Gen. Virol. 88, 3214–3223