Latency-associated nuclear antigen of Kaposi’s sarcoma-associated herpesvirus (human herpesvirus-8) binds ATF4/CREB2 and inhibits its transcriptional activation activity

Chunghun Lim, Hekwang Sohn, Yousang Gwack and Joonho Choe

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejeon 305-701, Korea

Introduction

Kaposi’s sarcoma-associated herpesvirus (KSHV), also called human herpesvirus-8, is the likely aetiological agent of Kaposi’s sarcoma (KS), the most common AIDS-related malignancy (Chang et al., 1994; Moore & Chang, 1995). KSHV also has been implicated in several B-cell lymphoproliferative diseases, including primary effusion lymphoma (PEL) (Cesarman et al., 1995), formerly designated body cavity-based lymphoma (BCBL), and some cases of multicentric Castleman’s disease (Soulier et al., 1995). KSHV establishes a latent infection in KS spindle cells, as well as PEL/BCBL cell lines. Among the viral genes expressed in the latent state, latency-associated nuclear antigen (LANA), encoded by ORF 73 of the KSHV genome, is a 222–234 kDa nuclear protein consisting of 1162 amino acids (Russo et al., 1996; Ganem, 1997; Cotter & Robertson, 1999). Recently, several cellular proteins have been shown to interact with LANA. These cellular proteins include histone H1 (Cotter & Robertson, 1999), RING3 (Platt et al., 1999) and p53 (Friborg et al., 1999).

We performed a yeast two-hybrid assay with full-length LANA as bait to identify cellular proteins that interact with LANA. Among several positive clones was a partial cDNA of activating transcription factor (ATF) 4 (Hai et al., 1989), also called cAMP response element (CRE)-binding protein (CREB) 2, a member of the ATF/CREB family of transcription factors, and represses the transcriptional activation activity of ATF4/CREB2. Repression by LANA is independent of the DNA-binding ability of ATF4/CREB2, since LANA also represses transactivation of ATF4/CREB2 fused to the GAL4 DNA-binding domain and does not affect the DNA-binding ability of ATF4/CREB2 in an electrophoretic mobility shift assay. The putative leucine zipper domain of LANA is required for binding to the relatively conserved basic region/leucine zipper domain (bZIP) of ATF4/CREB2, suggesting that the interaction may involve leucine zipper dimerization.

Latency-associated nuclear antigen (LANA), encoded by ORF 73 of Kaposi’s sarcoma-associated herpesvirus (KSHV; human herpesvirus-8), may play an important role in the persistence of the viral episome by tethering it to host chromosomes during mitosis. It also has been suggested from its amino acid sequence features that LANA may have transcription-regulatory activity. Here, it is reported that LANA interacts with activating transcription factor (ATF) 4/cAMP response element-binding protein (CREB) 2, a member of the ATF/CREB family of transcription factors, and represses the transcriptional activation activity of ATF4/CREB2. Repression by LANA is independent of the DNA-binding ability of ATF4/CREB2, since LANA also represses transactivation of ATF4/CREB2 fused to the GAL4 DNA-binding domain and does not affect the DNA-binding ability of ATF4/CREB2 in an electrophoretic mobility shift assay. The putative leucine zipper domain of LANA is required for binding to the relatively conserved basic region/leucine zipper domain (bZIP) of ATF4/CREB2, suggesting that the interaction may involve leucine zipper dimerization.

Author for correspondence: Joonho Choe.
Fax +82 42 869 5630. e-mail jchoe@mail.kaist.ac.kr
Through such cross-family dimerization, in addition to homo-/heterodimerization, they extend their DNA-binding specificity to allow differential regulation of transcription.

Here, we report that LANA interacts with ATF4/CREB2 in vivo and in vitro and that the bZIP domain of ATF4/CREB2 is required for the interaction. LANA represses the transcriptional activation activity of ATF4/CREB2 and the ATF4/CREB2-binding domain of LANA is required for repression. Since the bZIP domains of members of the ATF/CREB family have been shown to be targeted by many viral proteins, including adenovirus E1a (Liu & Green, 1994), human T-cell leukaemia virus Tax (Gachon et al., 1998; Bex & Gaynor, 1998) and hepatitis B virus pX (Maguire et al., 1991), LANA may represent a conserved strategy for transcriptional regulation during virus infection.

Methods

**Plasmids.** Total genomic DNA of KSHV was purified from a BCBL-1 cell line (Hirt lysis method, as described previously (Wilson & Patient, 1991). The 3.4 kb ORF 73 DNA was amplified by PCR and inserted into EcoRI/XhoI sites of pLexA (Clontech) and pGEX4T-1 (Amersham Pharmacia Biotech) to generate pLexA LANA and pGEX4T-1 LANA, respectively. To construct pEBG-LANA, a partially digested BamHI–NotI fragment of pGEX4T-1 LANA was cloned into pEBG, a eukaryotic expression vector encoding glutathione S-transferase (GST) (Mayer et al., 1995). pSG5 derivatives were constructed by inserting appropriate PCR products into the EcoRI/BglII sites of pSG5 (Stratagene) and they were designated pSG5 LANA(1–1162), pSG5 LANAAC(1–950) and pSG LANA-N(1–340) according to the size of their insert DNA fragment. For in vitro transcription and translation, pcDNA3 derivatives were made by inserting PCR products into the EcoRI/XhoI sites of pcDNA3 (Invitrogen) and were designated pcDNA3 LANA(1–1162), pcDNA3 LANA AC(1–950), pcDNA3 LANA NALZ(1–756), pcDNA3 LANA N(1–340), pcDNA3 LANA ADED(431–1162) and pcDNA3 LANA LZ-C(751–1162), according to the size of their DNA fragment. For green fluorescent protein (GFP)-fusion expression vectors, PCR products of LANA and deletion mutants were subcloned into the EcoRI/Sall sites of pEGFP-C1 (Clontech). For bacterial expression of the C-terminal 212 amino acids of LANA fused to maltose-binding protein (MBP), the corresponding PCR product was inserted into the EcoRI/Sall sites of pMAL-c2 (New England Biolabs). pATFx3-luc was made by inserting a PCR product containing three tandem ATF/CRE sites from pATFx3-CAT (Liang & Hai, 1997) into pGL2-Basic (Promega). pATFx3-CAT was a generous gift from T. Hai (Ohio State University, Columbus, OH, USA). pGEX CREB2, pGEX CREB2 bZIP and pGEX CREB2ALZIP were generous gifts from J. Mesnard (Gachon et al., 1998). To generate a haemagglutinin (HA)-tagged ATF4/CREB2 mammalian expression vector (pSRa ATF4/CREB2, ATF4/CREB2 cDNA amplified by PCR from pCG ATF4/CREB2 (Liang & Hai, 1997) was inserted into the Sall/Sall sites of pSRa. pCG ATF4/CREB2 was a generous gift from T. Hai. pM ATF4/CREB2, expressing ATF4/CREB2 fused to the C terminus of the GAL4 DNA-binding domain (DBD), was constructed by inserting ATF4/CREB2 cDNA into the EcoRI/Sall sites of pM.

**Yeast two-hybrid assay.** Yeast two-hybrid screening was performed as described previously (Lee et al., 1999), except that pLexA LANA was used as the bait plasmid and 10 μg of a B cell cDNA library in the yeast vector pGatrp was used for screening. Library plasmids containing positive clones were isolated (Lundblad, 1992), transformed into MC1061/P3 (Invitrogen) and sequenced by using the T7 Sequenase version 2.0 DNA sequencing kit (Amersham Pharmacia Biotech). Positive clones were confirmed by co-transformation with pLexA LANA into the yeast strain EGY048, harbouring the LacZ reporter plasmid p8op-lacZ (Clontech). More than two independent β-galactosidase assays were performed from a liquid culture using ONPG (Sigma) as a substrate (Lundblad, 1992).

**Cell culture, transfection and reporter assays.** HeLa and 293T cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and transfected by the calcium phosphate method (Kingston, 1992). Cells of 60–70% confluence were plated in 60 mm dishes at 12 h before transfection. The total amount of transfected DNA was adjusted with the appropriate blank vector. Cells were harvested at 36 h post-transfection. Luciferase and β-galactosidase assays were performed according to manufacturer's instructions (Promega) and the luciferase activity was normalized with co-transfected β-galactosidase activity. The results are the means of four independent experiments.

**In vivo binding assay.** HA-tagged ATF4/CREB2 expression vector (pSRa ATF4/CREB2) was co-transfected with either pEBG blank vector or pEBG LANA expression vector into 293T cells. At 36 h post-transfection, the cells were harvested and lysed in ice-cold PBS containing 0.5% Nonidet P-40 and a protease inhibitor cocktail (Roche Molecular Biochemicals) with brief sonication. After precipitating cell debris, the supernatant was incubated with glutathione–Sepharose 4B (Amersham Pharmacia Biotech) at 4 °C for 3 h. The beads were washed three times in PBS containing 0.1% Nonidet P-40 and a protease cocktail and then resuspended in a SDS-gel loading buffer. The eluted proteins were separated by 10% SDS–PAGE, transferred to nitrocellulose membrane and then immunoblotted and detected by ECL (Amersham Pharmacia Biotech).

**GST pull-down assay.** GST fusion proteins were incubated with [35S]methionine-labelled proteins synthesized by the Tnt T7 coupled transcription-translation reticulocyte lysate system, according to the manufacturer's instructions (Promega). After 3 h incubation at 4 °C in a binding buffer [20 mM Tris–HCl (pH 7.5), 50 mM NaCl, 2 mM MgCl2, 1 mM DTT and 0.1% Nonidet P-40], glutathione–Sepharose 4B beads were added and incubated further for 1 h at 4 °C. The beads were washed four times in binding buffer and resuspended in SDS-gel loading buffer. The eluted proteins were analysed by SDS–PAGE and subjected to autoradiography.

**Localization of GFP-fused LANA deletion mutants.** HeLa cells grown on coverslips were transfected with GFP or GFP-fused LANA deletion mutant expression plasmids by the calcium phosphate method, as described above. At 36 h post-transfection, the cells were washed three times and then fixed with formaldehyde (3.7%, v/v) in PBS. After two more washes, the coverslips were mounted and examined with a confocal laser scanning microscope (LSM510, Carl Zeiss).

**Production of rabbit anti-LANA polyclonal antiserum.** The C-terminal 212 amino acids of LANA fused to MBP were expressed in bacteria, purified and used in intradermal immunization. One month after the priming immunization with complete Freund’s adjuvant (Sigma), four additional boosting immunizations were administered with incomplete Freund’s adjuvant at intervals of 1 week. Rabbit anti-LANA polyclonal antiserum diluted 1:500 was used in Western blots of total cell lysates.

**Electrophoretic mobility shift assay (EMSA).** Synthetic oligonucleotides containing CRE (5′- AAGATTGCCTCAGCTCAGAGCTTAG 3′) were labelled with [γ-32P]ATP by 14 poly nucleotide kinase and annealed with the complementary strand. Labelled probe (~ 100
KSHV LANA represses transactivation of ATF4

Results

Yeast two-hybrid assay

Full-length LANA fused to the LexA DNA-binding domain, which showed no autonomous activation of the leu2 gene under the control of LexA operators in yeast, was used as bait for a yeast two-hybrid assay to identify cellular proteins that could interact with LANA. A B cell cDNA library fused to the B42 activation domain was screened. From $3.5 \times 10^5$ transformants, 26 positive clones were classified into seven groups according to their restriction enzyme pattern and DNA sequence analysis. Sequence analysis revealed the following: six clones were partial cDNA s of ribosomal protein S28; one clone was RING3, previously identified as a LANA-interacting protein by using the GAL4 yeast two-hybrid system (Platt et al., 1999); and six clones were ATF4/CREB2. We focused on ATF4/CREB2 because LANA has been thought to have transcription-regulatory activity (Russo et al., 1996; Ganem, 1997; Cotter & Robertson, 1999). We identified a full-length cDNA of ATF4/CREB2 from a yeast two-hybrid that encoded the bZIP domain of ATF4/CREB2, a relatively conserved domain among members of the ATF/CREB family of transcription factors (Sassone-Corsi, 1995). In order to confirm the interaction between LANA and ATF4/CREB2, the isolated library plasmid was co-transformed with pLexA LANA into the yeast strain EGY048, harbouring the LacZ reporter plasmid p8op-lacZ. Next, the yeast strain EGY048, harbouring the LacZ reporter plasmid library plasmid was co-transformed with pLexA LANA into interaction between LANA and ATF4 transcription factors (Sassone-Corsi, 1995). In order to confirm the cDNA of ATF4 1997; Cotter & Robertson, 1999). We identified a partial sequence analysis. Sequence analysis revealed the following: six clones were partial cDNAs of ribosomal protein S28; one sequence according to their restriction enzyme pattern and DNA sequence analysis revealed the following: six clones were partial cDNAs of ribosomal protein S28; one clone was RING3, previously identified as a LANA-interacting protein by using the GAL4 yeast two-hybrid system (Platt et al., 1999); and six clones were ATF4/CREB2. We focused on ATF4/CREB2 because LANA has been thought to have transcription-regulatory activity (Russo et al., 1996; Ganem, 1997; Cotter & Robertson, 1999). We identified a full-length cDNA of ATF4/CREB2 from a yeast two-hybrid that encoded the bZIP domain of ATF4/CREB2, a relatively conserved domain among members of the ATF/CREB family of transcription factors (Sassone-Corsi, 1995). In order to confirm the interaction between LANA and ATF4/CREB2, the isolated library plasmid was co-transformed with pLexA LANA into the yeast strain EGY048, harbouring the LacZ reporter plasmid p8op-lacZ. Next, β-galactosidase assays were performed on a liquid culture. When both plasmids were co-transformed, more than 50-fold activation of β-galactosidase activity was observed, compared with transformants harboring pLexA LANA alone or pLexA lamin instead of pLexA LANA as a negative control (data not shown).

Assay of binding in vivo between LANA and ATF4/CREB2

In order to confirm that LANA interacts with ATF4/CREB2 in mammalian cells, an in vivo binding assay was performed. Plasmids expressing GST or GST–LANA were co-transfected with HA-tagged ATF4/CREB2 expression plasmid into 293T cells. After cells were harvested, proteins were precipitated with glutathione–Sepharose beads and immunoblotted by using a monoclonal antibody against GST (Fig. 1a). In Fig. 1(b), total cell lysates (lane T) or glutathione-bound proteins (lane P) were immunoblotted by using a monoclonal antibody against HA. Although HA-tagged ATF4/CREB2 protein was expressed at comparable levels (lanes 3 and 5), it was only co-precipitated with GST–LANA protein (lane 6). When the GST–LANA expression plasmid alone (lane 2) or GST- and HA–ATF4/CREB2-expressing plasmids (lane 4) were co-transfected, no such band was observed, confirming that the pulled-down protein was not a non-specific protein of similar size. This result shows that LANA associates with ATF4/CREB2 in vivo.

In vitro binding assay and mapping of the binding domains in LANA and ATF4/CREB2

In order to define the binding domains of LANA and ATF4/CREB2, we carried out an in vitro GST pull-down assay. Various deletion mutants of ATF4/CREB2 and LANA used in this assay are shown in Fig. 2(a, c). GST fused-ATF4/CREB2 and deletion mutant proteins were expressed in bacteria and purified by using glutathione–Sepharose beads. The amounts of GST-fused proteins used in the GST pull-down assay were comparable in a Coomassie blue-stained gel (Fig. 2b). Fig. 2(c) shows that the bZIP domain of ATF4/CREB2 is necessary and sufficient for binding to in vitro-translated, radiolabelled LANA. LANA deletion mutants containing a putative leucine zipper domain were precipitated with GST–ATF4/CREB2 fusion protein immobilized on glutathione–Sepharose beads (Fig. 2c). GST–ATF4/CREB2 bZIP fusion proteins showed a higher affinity for LANA than did the wild-type ATF4/CREB2. The above results show that LANA binds ATF4/CREB2 in vivo.

Effect of LANA on transcriptional activation by ATF4/CREB2

Next, we examined whether LANA could modulate the transcriptional activation activity of ATF4/CREB2. When co-transfected into HeLa cells with a luciferase reporter plasmid containing three tandem ATF/CRE sites (pATFx3-luc), ATF4/CREB2 activated transcription of the reporter plasmid (Li and Hai, 1997). Fig. 3(a) shows that increasing amounts of LANA repressed the transactivation activity of ATF4/CREB2 on the ATF/CRE promoter in a dose-dependent manner. However, pSG5 LANA-N, which does not contain the binding domain for ATF4/CREB2, could not repress the transactivation activity of ATF4/CREB2. This finding indicates that repression by LANA requires an ATF4/CREB2-binding domain. Since LANA may repress the transactivation activity of ATF4/CREB2 by binding to the bZIP domain, which is required for dimerization/DNA binding, and affecting the DNA-binding ability of ATF4/CREB2, we adopted the GAL4 fusion system to exclude this possibility. The use of the GAL4 system simplifies the investigation of the functional interaction between LANA and ATF4/CREB2, since other members of the ATF/CREB family of transcription factors can also bind the same ATF/CRE sites (Hai et al., 1989). GAL4-fused ATF4/CREB2 was constructed and a luciferase reporter containing

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 19 Jan 2019 00:48:01
five GAL4-binding sites (pFR-luc) was used as a reporter plasmid. As shown in Fig. 3(b), pFR-luc was activated by GAL4–ATF4/CREB2 and its transactivation was repressed by LANA in a dose-dependent manner. Repression of ATF4/CREB2 transactivation activity by LANA is also dependent on the presence of a binding domain for ATF4/CREB2. Re-
pression by LANA was not cell type specific, as a similar result was obtained with 293T cells (data not shown).

The cellular localization of LANA and C-terminal deletion mutants was examined in order to confirm that the inability of the LANA deletion mutant to repress the transactivation activity of ATF4/CREB2 was not due to the cellular localization of LANA but was due to the absence of an ATF4/CREB2-binding domain. As shown in Fig. 4, LANA and all C-terminal deletion mutants fused to GFP were localized in the nucleus, which is consistent with the results of a previous report (Friborg et al., 1999). Interestingly, an N-terminal deletion mutant of LANA (amino acids 324–1162) designated as LANA ΔN was localized exclusively in the cytoplasm (Fig. 4f). This exclusively cytoplasmic localization of LANA ΔN was not due to aberrant expression or a frameshift between GFP and LANA. Expression of LANA ΔN with the correct size was demonstrated by a Western blot of a total cell lysate transfected with the indicated expression vectors by using a rabbit polyclonal antiserum against the C terminus of LANA (Fig. 4g). These findings imply that the N terminus of LANA (1–324) may contain the nuclear localization signal (NLS). Taken together, these results suggest (i) that LANA inhibits the transcriptional activation activity of ATF4/CREB2 specifically by binding to the bZIP domain and (ii) that the ATF4/CREB2-binding domain of LANA is necessary for repression of the transactivation activity of ATF4/CREB2.

**Effect of LANA on the DNA-binding ability of ATF4/CREB2**

Although LANA repressed the transcriptional activation activity of GAL4–ATF4/CREB2, we cannot exclude the possibility that LANA affects the DNA-binding ability of ATF4/CREB2 and thereby represses the transcriptional activation activity of ATF4/CREB2 on a reporter promoter containing ATF/CRE. Therefore, we examined the effect of

![Fig. 3. Repression by LANA of ATF4/CREB2-dependent transcriptional activation](image)

HeLa cells were co-transfected with 1–5 µg pATFx3-luc, 2 µg pCG or pCG ATF4/CREB2 and indicated amounts of pSG5 derivatives expressing LANA deletion mutants (a) or 1–8 µg pFR-luc, 1 µg pM or pM ATF4/CREB2 and indicated amounts of pSG5 derivatives expressing LANA deletion mutants (b). Relative luciferase activity (RLU) was normalized to co-transfected β-galactosidase activity (β-gal). Each result is the mean of four independent experiments and standard deviations are indicated by error bars.

![Fig. 4. Cellular localization of LANA and deletion mutants](image)

HeLa cells were transfected with pEGFP-C1 vector (a), pEGFP LANA (b), pEGFP LANA ΔC (c), pEGFP LANA NALZ (d), pEGFP LANA N (e) or pEGFP LANA ΔN (f). Localization patterns of GFP-fusion proteins were examined at 36 h post-transfection. (g) Western blot of total cell lysate transfected with indicated expression vectors, probed with rabbit anti-LANA polyclonal antiserum.
LANA in EMSA with GST–ATF4/CREB2. As shown in Fig. 5, when labelled probe containing the CRE sequence was incubated with GST–ATF4/CREB2, two major shifted bands were observed (lane 3, C1 and C2). The addition of a 10-fold excess of unlabelled oligonucleotides containing a CRE- or Oct-1-binding site was used in competition reactions. IVT mock, Control reticulocyte lysate; IVT LANA, in vitro-translated LANA.

**Discussion**

In this report, we found that KSHV LANA interacts with ATF4/CREB2, a member of the ATF/CREB family of transcription factors, by using the yeast two-hybrid assay. The interaction was confirmed by an in vitro GST pull-down assay and in vivo co-precipitation of the two proteins. The putative leucine zipper domain of LANA and the relatively conserved bZIP domain of ATF4/CREB2, responsible for DNA-binding/dimerization of the ATF/CREB family, are required for binding.

In transient co-transfection experiments, LANA repressed transcriptional activation of the reporter promoter by ATF4/CREB2. The ATF4/CREB2-binding domain in LANA was necessary for repression, implying that LANA may exert a transcription-inhibitory effect via direct binding to ATF4/CREB2. Since LANA inhibited transcriptional activation of the promoter by a GAL4–ATF4 fusion protein and did not affect the DNA-binding ability of ATF4/CREB2 under our experimental conditions, another mechanism may be involved in the process of repression by LANA. However, the possibility that LANA modulates DNA binding of ATF4/CREB2 in the context of a promoter cannot be excluded.

The PSORT II program (http://psort.nibb.ac.jp/) predicted three putative NLSs in the published amino acid sequence of LANA (GenBank accession no. AAC57158). These were PRRKH (amino acids 43–47), RKRR (amino acids 200–203) and PGVRMR (amino acids 995–1001). The two N-terminal putative deletion mutants of LANA fused to GFP localized exclusively in the nucleus, but an N-terminal deletion mutant lacking these motifs (LANA ΔN) did not. Also, we found that the N-terminal 213 amino acids of LANA were necessary and sufficient for exclusively nuclear localization and that the C-terminal 212 amino acids of LANA were also necessary for nuclear localization in an N-terminal deletion mutant of LANA, which showed a similar localization pattern to that of a GFP control (data not shown).

Three models of active repression have been defined (Galvin & Shi, 1997). From our results, we deduced that LANA belongs by definition to the type II quenching repressors; that is, a protein that represses transcription by interacting with the activator itself. In order to elucidate the repression mechanism of LANA, we examined whether LANA acted as a direct repressor tethered to a promoter by using the GAL4–LANA fusion system. GAL4–LANA repressed a thymidine kinase promoter containing tandem GAL4-binding sites weakly in a dose- and GAL4 DBD-dependent manner (data not shown). Because the expression level of GAL4–LANA in our study was relatively low, we could not determine the reason for the observed weak repression. We also tested whether LANA recruited a co-repressor complex containing histone deacetylase activity (Knoepfler & Eisenman, 1999) to the promoter via ATF4/CREB2. LANA did not interact directly with GST-fused histone deacetylases 1, 2 and 3 in an in vitro GST pull-down assay. The repression of LANA upon transcriptional activation by GAL4–ATF4/CREB2 was not relieved by treatment with trichostatin A, a histone deacetylase inhibitor (Johnson & Turner, 1999), in a transient co-transfection experiment (data not shown). The above result indicated that the repression of LANA is not related to histone deacetylase activity.

Moderate repression of ATF4/CREB2-dependent transcription by LANA can be explained by multiple interactions of ATF4/CREB2 with general transcription factors and CBP (Liang & Hai, 1997). The C-terminal bZIP domain of
ATF4/CREB2, in addition to DNA binding/dimerization, has the ability to interact with general transcription factors/CBP and activates transcription weakly. However, LANA may be unable to inhibit the transactivation activity of ATF4/CREB2 completely because the N terminus of ATF4/CREB2 can also interact with general transcription factors and still activates transcription more efficiently than the C-terminal bZIP domain.

The physiological functions of ATF4/CREB2 have not been defined. It can repress protein kinase A-dependent transcriptional activation (Karpinski et al., 1992) or activate transcription when tethered to a promoter by a GAL DBD fusion or via ATF4/CREB2-binding sites (Liang & Hai, 1997). A previous study reported that LANA shows preferential binding to different regions of KSHV DNA in vitro (Cotter & Robertson, 1999). However, it is uncertain whether LANA binds directly to DNA with sequence preference, because in vitro-translated LANA used in a DNA-binding assay may be contaminated with cellular proteins in a rabbit reticulocyte lysate. LANA may use sequence-specific DNA-binding adapter proteins, such as ATF4/CREB2, for sequence-preferential DNA binding or may target a viral or cellular promoter to regulate a transcriptional network during virus infection. It would be interesting to investigate a viral or cellular promoter targeted by LANA via ATF4/CREB2, in addition to the repression mechanism of LANA.

This work was supported in part by grants from the National Research Laboratory Program of the Korea Institute of Science and Technology Evaluation and Planning (KISTEP), the Korea Science and Engineering Foundation (KOSEF) through the Protein Network Research Center at Yonsei University and the BK21 Program of the Ministry of Education.

References


associated herpesvirus (human herpesvirus 8) is encoded by orf73 and is a component of the latency-associated nuclear antigen. *Journal of Virology* **71**, 5915–5921.


Received 19 April 2000; Accepted 14 July 2000