JOURNAL OF VIROLOGY, Oct. 1995, p. 6005–6009 Vol. 69, No. 10
0022-538X/95/$04.00+0

Identification of an NF-κB Binding Site in the Bovine Leukemia Virus Promoter

PATRICIA A. BROOKS,[1]* JENNIFER K. NYBORG,[2] AND GARY L. COCKERELL[1]

Department of Pathology[1] and Department of Biochemistry and Molecular Biology,[2] Colorado State University, Fort Collins, Colorado 80523

Received 5 December 1994/Accepted 29 June 1995

Although the mechanism by which bovine leukemia virus (BLV) induces neoplastic transformation of the host B cells is unknown, it is likely that critical interactions between cellular DNA-binding proteins and the virus are involved. We have used DNase I protection (footprinting) assays to construct a map of protein-DNA interactions on the 5′ long terminal repeat of BLV. In addition to the three cyclic AMP response elements previously reported, we have also found an NF-κB binding site between −118 and −70 nucleotides upstream of the RNA start site. This site binds several members of the κB family of proteins, including p49, p50, and p65, in both footprint and electrophoretic mobility shift assays and functions as an enhancer element when inserted upstream of the chloramphenicol acetyltransferase gene. NF-κB may be a critical nuclear binding protein that regulates both viral replication and key cellular genes in BLV-infected B cells.

Bovine leukemia virus (BLV) is a prevalent, naturally occurring oncogenic retrovirus of cattle. Cattle become persistently infected yet often remain clinically normal. However, up to 30% of cattle eventually develop a preneoplastic condition, termed persistent lymphocytosis, and of these animals, approximately 10% develop overt leukemia and/or lymphomas (25). On the basis of structural and biological similarities, BLV is classified with the human T-cell leukemia virus type I (HTLV-I) and type II (HTLV-II) in the *Oncovirinae* subfamily of *Retroviridae* (16, 41). The mechanism of neoplastic transformation by these viruses is incompletely understood. The viruses do not contain a known oncogene, nor do they preferentially integrate into a specific region of the host genome (27). However, viral replication and host cell activation are transcriptionally linked. Peripheral blood lymphocytes from BLV-infected animals with persistent lymphocytosis contain low or undetectable levels of virus when first isolated (24, 28). Within 4 h of in vitro culture, the lymphocytes begin to spontaneously proliferate in the absence of added mitogens, and viral mRNA can be detected (8). This positive cycle of costimulation may be the first step in the neoplastic transformation of host cells.

The viral *trans*-activating protein, Tax, appears to be critical in these transcriptional events. Rather than binding directly to DNA, Tax appears to act via cellular DNA-binding proteins which bind to the viral long terminal repeat (LTR). HTLV-I Tax has been shown to enhance binding activity of several nuclear proteins which regulate the transcription of genes critical to the host cell activation and proliferation (2, 11). These include the activating transcription factor/cyclic AMP (cAMP) response element binding protein (ATF/CREB) family of proteins, oncoproteins such as Fos-Jun and serum response factor (15). BLV Tax also acts via cellular DNA-binding proteins on the BLV LTR (9), but its range of protein interactions is poorly understood.

The ATF/CREB binding sites and the binding of CREB protein have been extensively characterized in the BLV LTR (10, 26, 45). However, identification of other key regulatory DNA-binding proteins that interact with the BLV LTR is critical to understanding the link between viral and host cell transcriptional activation. To identify the full range of transcriptional activators involved in viral and host cell stimulation, we began mapping the DNA-protein interactions on the BLV LTR. We report here a site in the BLV LTR between the second and third cAMP response element (CRE) sites that binds NF-κB, a member of the Rel family of transcriptional regulatory proteins.

MATERIALS AND METHODS

Nuclear extracts. Crude nuclear extracts were prepared from freshly isolated lymphocytes from the following animals: two naturally infected, asymptomatic, BLV-seropositive cows (lymphocyte extracts 1 and 2); one BLV-seronegative cow; and one experimentally infected BLV-seropositive sheep. In addition, nuclear extracts were prepared from BLV-bat (19), a BLV-infected cell line derived from Tb1Lu bat lung cells (kindly provided by Craig Dees, Oakridge National Laboratory), and 81C (14), a feline kidney cell line; these represented nonlymphoid BLV-positive and BLV-negative cells, respectively, for comparison to BLV-positive and BLV-negative lymphocyte extracts. All cells were washed twice with phosphate-buffered saline, and crude nuclear protein extracts were prepared as previously described (12). Protein concentrations were determined by using a modified Bradford assay (7).

Purified DNA-binding proteins. Human CREB A was expressed in *Escherichia coli* (cDNA was kindly provided by Mark Gilman, Cold Spring Harbor Laboratory) and purified as previously described (5, 22). NF-κB p50 (amino acids 1 to 377) and NF-κB p65 (amino acids 1 to 309) were purified by Ni^{2+} chelate chromatography as described previously (40). NF-κB p49 and SP-1 were obtained from Promega Corporation (Madison, Wis.). The serum response factor was expressed in *E. coli*, purified by ammonium sulfate precipitation (15% [wt/vol]) of sonicated lysates, and dialyzed against Superdex buffer (21).

DNA probes. The 5′ LTR of BLV (41) in a pUC18 plasmid was used to prepare probes for footprinting. Plasmids were opened at the 5′ end of the probe sequence, dephosphorylated, and digested to produce a 703-bp sequence that could be singly end labeled with γ-^{32}P (Fig. 1). A synthetic 53-bp complementary doubled-stranded oligonucleotide of the NF-κB binding site in the BLV LTR (NFLTR) and a 22-bp double-stranded oligonucleotide containing the NF-κB consensus sequence (from the mouse κ gene promoter; Promega Corp.) were end labeled with ^{32}P for electrophoretic mobility shift assays (EMSA). NF-κB and CRE consensus oligonucleotides were used as competitive DNA targets. The nucleotide sequences of the top strands were 5′-GATCTGGCTAGAATC CCCGTACCTCCCCAACTTCCCCTTTCCCGAAAAATCCA-3′ for NFLTR, 5′-AGTTGAGGGGACTTTCCCAGGC-3′ for the NF-κB consensus oligonucleotide, and 5′-GATCTCCATGACGTCAATTGA-3′ for the CRE consensus sequence (from the human chorionic gonadotropin promoter).

DNase I protection (footprinting) assays. DNase I footprinting assays were performed as previously described (36). Briefly, the BLV LTR 703-bp probe was 5′ end labeled with ^{32}P at a position 216 nucleotides upstream of the BLV RNA start site. A 50-μl binding reaction mixture containing 0.02 nM DNA probe plus

* Corresponding author. Mailing address: Department of Pathology, Colorado State University, Fort Collins, CO 80523. Phone: (970) 491-5106. Fax: (970) 491-0603. Electronic mail address: pbrooks@vines.colostate.edu.

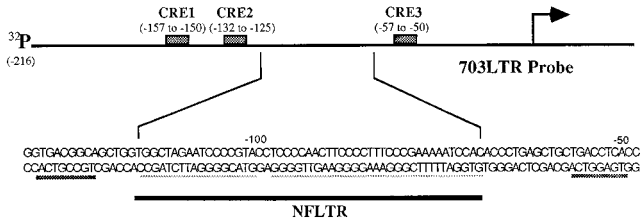

FIG. 1. Schematic representation of BLV LTR probes. The 703-bp LTR (703LTR) probe, which starts 216 bp upstream from the RNA start site (bent arrow; U3/R junction, proviral position −216), was singly end labeled with ^{32}P for use in DNase I protection assays. The sequence denotes the region between the second and third CRE sites (hatched boxes) where the newly observed NF-κB protein-binding site is located. The synthetic double-stranded oligonucleotide probe (NFLTR) used in EMSA is indicated by the heavy line under the sequence of NFLTR in the coding strand. The dotted line indicates the region of DNase I protection shown in subsequent footprinting assays (Fig. 2 to 4).

200 ng of alternating copolymer poly[dI-dC] · poly[dI-dC] and crude nuclear extracts or purified proteins in 25 mM Tris-HCl (pH 7.9)–50 mM KCl–6.25 mM $MgCl_2$–0.5 mM EDTA–10% (vol/vol) glycerol–10 mM dithiothreitol (0.1 M TM buffer) was made. The mixture was incubated on ice for 15 to 30 min, partially digested with DNase I, purified, and electrophoresed in denaturing 6.5% polyacrylamide gels.

EMSA. The 53-bp oligonucleotide (NFLTR) containing the putative BLV LTR NF-κB binding site and the κB consensus oligonucleotide were end labeled with ^{32}P and incubated with purified proteins or crude nuclear extracts. Binding reaction mixtures contained the DNA-binding protein in 0.1 M TM buffer–0.5 nM poly[dI-dC] · poly[dI-dC]–0.02 nM end-labeled probe. The binding reaction mixtures were incubated on ice for 1 h, analyzed on 5% nondenaturing polyacrylamide gels, dried, and autoradiographed.

CAT plasmids. The parental chloramphenicol acetyltransferase (CAT) plasmids were obtained from Promega Corp. pCATP-NFLTR was derived from the pCAT-promoter vector (pCAT-P) in which three tandem inserts of the NFLTR oligonucleotide were cloned into the *Bgl*II site upstream of the simian virus 40 (SV40) promoter. The nucleotide sequence of the pCAT-P–NFLTR was determined by using the Sequenase DNA sequencing kit (United States Biochemical Corp.) to verify the number of inserts and their orientations. The pCAT-control vector (pCAT-C) containing both SV40 promoter and enhancer sequences was used as a positive control in the transfection experiments.

In vivo transcription assays (CAT assays). CV-1 (ATCC CCL70) cells were propagated in RPMI 1640 supplemented with 10% fetal calf serum. Subconfluent cells were transfected with 5 μg of either pCAT-P, pCAT-P–NFLTR, or pCAT-C, by using LipofectAMINE (Life Technologies, Inc., Gaithersburg, Md.). To stimulate NF-κB production, cells were treated with phorbol 12-myristate 13-acetate (PMA) at 50 ng/ml in the medium at 56 h posttransfection (39). Cells were harvested 16 h later and lysed in 0.25 M Tris-HCl buffer by three successive rapid freeze-thaw cycles. Extracts were assayed for CAT enzyme activity as previously described (18). CAT activity was detected by thin-layer chromatography and autoradiography, and CAT enzyme activity was quantitated by PhosphorImage (Molecular Dynamics, Sunnyvale, Calif.) analysis.

RESULTS

The BLV LTR contains an NF-κB binding site. Previous studies have shown that the viral Tax protein regulates viral transcription indirectly through cellular proteins that bind to the three 21-bp repeat elements in the HTLV-I and HTLV-II LTRs (37). To begin mapping the BLV LTR to identify nuclear proteins which control viral replication, we started with a purified human protein, CREB A (5), which was likely to bind to the three 21-bp repeats previously identified in the BLV LTR. CREB A bound to the three imperfect CRE sites located at −157 to −150, −132 to −125, and −57 to −50 (Fig. 2). These sites have a common 8-bp core sequence, TGACGTCA, which interacts with multiple regulatory proteins (22, 23). In repeated experiments the footprint over the CRE closest to the cap site was less consistent than those of the other two, suggesting weaker CREB binding. This may explain the previous observation that the first CRE upstream of the RNA start site does not confer significant Tax responsiveness (9).

To characterize other nuclear binding proteins that selectively bind the BLV LTR, we used crude nuclear extracts from freshly isolated bovine lymphocytes of BLV-seropositive cows and from BLV-infected nonlymphocytic cell lines. Examination of the protein-DNA interactions produced by these extracts revealed several distinct and unique footprints on the BLV LTR. In particular, extracts from both bovine lymphocytes and BLV-bat cells produced a consistent, strong footprint between the second and third CREs upstream of the cap site (Fig. 2). Binding of the proteins conferred DNase I protection between −118 to −70, with a small incompletely protected site at approximately −100 to −98. Given the size of the site and the incompletely protected area, this probably represents two binding sites. Similar protection was seen with lymphocyte nuclear extracts from a BLV-seronegative cow but not with nuclear extracts from a nonlymphocyte cell line, 81C (14) (data not shown).

Since the sequence of this protected site showed some similarity to the NF-κB consensus sequence 5′-GGGRNNYYCC-3′, we next examined footprints produced by purified NF-κB proteins. We first examined the binding pattern of a recently described member of the κB family, p49 (also referred to as p52 [4]). This protein binds as a homodimer or heterodimer and acts synergistically in transcription with at least one other member of the NF-κB family, p65 (43). The p49 produced DNase I protection indistinguishable from that of the unknown proteins in the crude nuclear extracts and conferred DNase I protection even at low concentrations (Fig. 3A).

To determine the specificity of the putative κB site, we included unlabeled double-stranded oligonucleotides of either a specific κB consensus sequence or an unrelated sequence in the reaction mix. Binding was competitively inhibited by the addition of the NF-κB consensus oligonucleotide but not with an unrelated DNA sequence, such as the CRE consensus oligonucleotide, indicating that the site on the BLV LTR was specific for NF-κB (Fig. 3B).

Since p49 is only one of several reported members of the

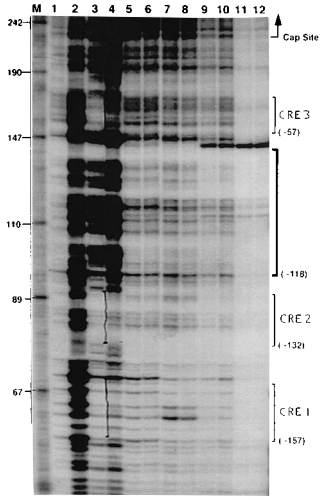

FIG. 2. DNase I footprints on BLV proviral LTR after incubation with CREB or crude nuclear extracts. For each lane, the 703-bp probe was incubated with 7 μg of protein, unless otherwise noted. All samples were run in duplicate in adjacent lanes. The brackets on the right indicate regions of protection, and the numbers in parentheses indicate the number of base pairs upstream of the RNA start site. End-labeled DNA size markers (pUC18 digested with *Hpa*II) were run in the first lane for reference (the numbers on the left indicate fragment sizes [in base pairs]). Lanes: M, pUC18-*Hpa*II fragments; 1 and 2, no protein; 3 and 4, CREB (4 μg); 5 and 6, bovine lymphocyte extract no. 1; 7 and 8, BLV-bat extract; 9 and 10, bovine lymphocyte extract no. 2; 11 and 12, bovine lymphocyte extract no. 2 (14 μg).

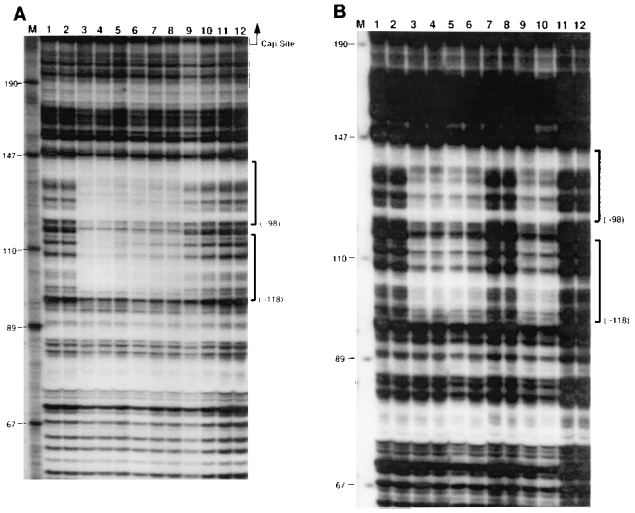

FIG. 3. DNase I footprint of BLV proviral LTR after incubation with NF-κB p49. The brackets on the right indicate areas of protection, and lane M contained pUC18-*Hpa*II fragments, as described in the legend to Fig. 2. (A) For each lane, the 703-bp probe was incubated with various amounts of p49 protein. Lanes: 1 and 2, no protein; 3 and 4, 0.20 μg; 5 and 6, 0.10 μg; 7 and 8, 0.05 μg; 9 and 10, 0.01 μg; 11 and 12, no protein. (B) For each lane, the DNA probe was incubated with 0.10 μg of p49 with or without unlabeled double-stranded oligonucleotides as DNA-binding competitors. Lanes: 1 and 2, no protein; 3 and 4, p49 only; 5 and 6, p49 plus 0.02 nM NF-κB consensus sequence (1:1 with probe); 7 and 8, p49 plus 2.0 nM NF-κB consensus sequence (100:1 with probe); 9 and 10, p49 plus 2.0 nM CREB consensus sequence (100:1 with probe); 11 and 12, no protein.

NF-κB/Rel family of proteins, we next examined the binding of two other proteins, p50 and p65. Purified NF-κB p50 produced a footprint essentially identical to that of p49 (Fig. 4); purified p65 did not appear to bind as a homodimer under the conditions in the footprint assay but did bind as a homodimer in EMSA (see below).

The BLV LTR κB site binds NF-κB proteins in EMSA. We next examined the isolated NF-κB binding site in EMSA to define which proteins in the nuclear extracts were binding to

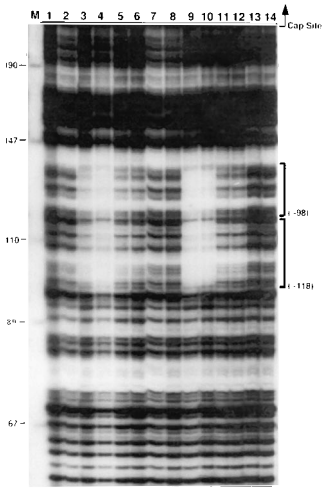

FIG. 4. DNase I footprints on BLV proviral LTR after incubation with different NF-κB proteins (brackets on the right indicate areas of protection, and lane M contained pUC18-*Hpa*II fragments, as described in the legend to Fig. 2). Lanes: 1 and 2, no protein; 3 and 4, p49 (0.1 μg); 5 and 6, p50 (4 μg); 7 and 8, p65 (4 μg); 9 and 10, p49 (0.1 μg) plus p65 (4 μg); 11 and 12, p50 (4.4 μg) plus p65 (4 μg); 13 and 14, no protein.

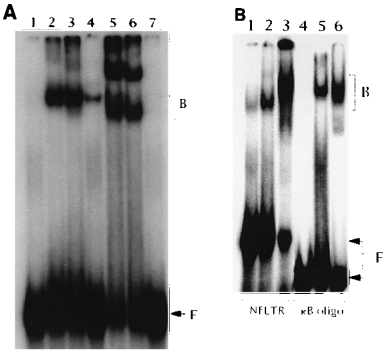

FIG. 5. EMSA of BLV proviral NF-κB binding site (NFLTR) or κB consensus oligonucleotide (κB oligo). B, bound probe; F, free probe. (A) Binding of purified proteins on NFLTR: Lanes: 1, no protein; 2, p49 (0.3 μg); 3, p49 (0.3 μg) plus p65 (4 μg); 4, p50 (2 μg); 5, p50 (2 μg) plus p65 (4 μg); 6, p65 (4 μg); 7, p65 (4 μg) plus 2.0 nM NF-κB consensus sequence (100:1 molar ratio with probe). (B) Comparison of protein binding on NFLTR or κB consensus oligonucleotide by using crude nuclear extracts or purified protein. Lanes 1, no protein; 2, p49 (0.3 μg); 3, ovine lymphocyte nuclear extract (12 μg); 4, no protein; 5, p49 (0.3 μg); 6, ovine lymphocyte nuclear extract (12 μg).

the κB site in the BLV LTR. The 53-bp oligonucleotide encompassing the putative κB site (NFLTR) found in the BLV LTR was tested for NF-κB binding activity in an EMSA using purified p49, p50, and p65. As shown in Fig. 5A, all three proteins recognized the κB site in the BLV LTR. Binding could be competitively inhibited with the NF-κB consensus oligonucleotide, and the addition of a nonspecific protein such as CREB did not result in a shift (data not shown). The radiolabeled NFLTR and κB consensus oligonucleotides were then incubated with crude nuclear extracts from a BLV-infected sheep. Even at very low crude nuclear extract concentrations, most of the NFLTR was completely shifted and did not enter the gel (Fig. 5B). The nuclear extracts tested show a specific DNA-protein shift when a radiolabeled κB consensus sequence was used as the DNA target. The addition of an unlabeled NFLTR probe resulted in complete loss of binding to the radiolabeled κB consensus sequence, whereas the addition of up to a 100:1 molar ratio of a nonspecific DNA sequence, such as the CRE consensus sequence or linearized pUC18 plasmid, had no effect on sequence-specific binding to the κB consensus (data not shown).

The BLV LTR κB site acts as an enhancer element. To determine if the κB binding site in the BLV LTR could act as a functional enhancer, the BLV LTR κB binding site was inserted upstream of the SV40 promoter in the plasmid pCAT-P (Fig. 6A). Transient transfection of this pCAT-P–NFLTR plasmid into CV-1 cells resulted in a 4.5-fold increase in CAT activity compared with that of the pCAT-P plasmid in the same cell line. However, treatment of the transfected cells with PMA to upregulate expression of NF-κB resulted in a 29-fold increase in CAT expression (Fig. 6B). The positive control, pCAT-C, which contains an NF-κB site in the SV40 enhancer region, also responded to PMA stimulation, whereas the negative control, pCAT-P, showed little change in activity.

DISCUSSION

The NF-κB enhancer element was first identified in the gene for the immunoglobulin light chain kappa and has since been found to regulate a wide variety of genes, including those for interleukin 2 (IL-2), IL-4, and IL-6; the IL-2 receptor α (IL-2Rα) chain; nitric oxide synthetase; granulocyte-macrophage colony-stimulating factor; and serum amyloid A (6, 13, 29, 31, 46). The coordinated induction of genes in B cells activated by

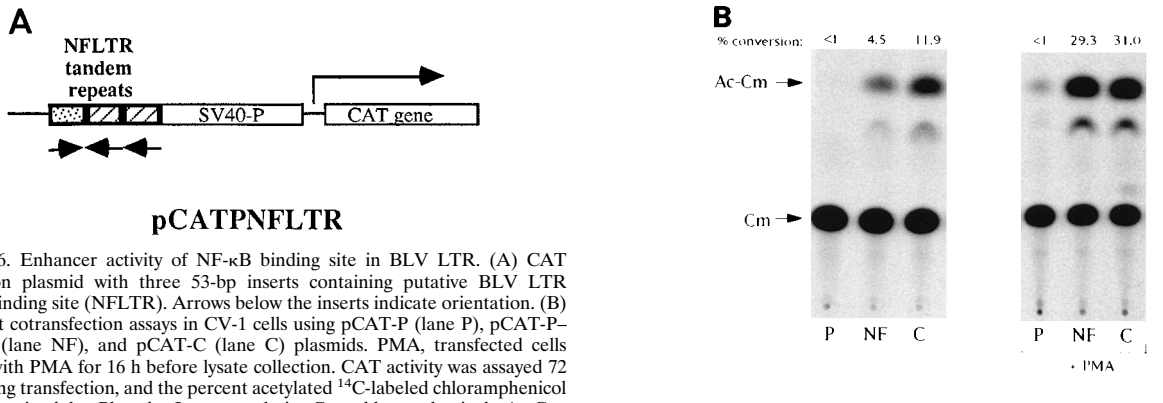

FIG. 6. Enhancer activity of NF-κB binding site in BLV LTR. (A) CAT expression plasmid with three 53-bp inserts containing putative BLV LTR NF-κB binding site (NFLTR). Arrows below the inserts indicate orientation. (B) Transient cotransfection assays in CV-1 cells using pCAT-P (lane P), pCAT-P–NFLTR (lane NF), and pCAT-C (lane C) plasmids. PMA, transfected cells treated with PMA for 16 h before lysate collection. CAT activity was assayed 72 h following transfection, and the percent acetylated ^{14}C-labeled chloramphenicol was determined by PhosphorImage analysis. Cm, chloramphenicol; Ac-Cm, acetylated chloramphenicol.

lipopolysaccharide has recently been shown to be mediated by NF-κB (33). The κB consensus site has also been found in the LTR of human immunodeficiency virus type 1, and the HTLV-I *trans*-activating protein Tax can activate human immunodeficiency virus replication via this enhancer element (34). An NF-κB binding site has been reported in the HTLV-I LTR between the second and third CREs (35) but was not associated with the classical κB consensus sequence GGGRN NYYCC.

We report here the presence of an NF-κB enhancer element between the second and third CREs in the BLV LTR (Fig. 7). NF-κB is constitutively expressed in B cells, the host cell for BLV (32, 38), which is consistent with our finding that DNase I protection is conferred with both BLV-infected and noninfected bovine lymphocytes. This constitutive expression could provide a low level of transcriptional activity, which is then upregulated when the cell is immunologically activated by a second, unrelated event. Since only a small proportion of circulating B cells are infected in the preclinical stages of BLV infection, progression of the disease may depend on an unrelated activation event which stimulates rare BLV-infected B cells. Once such cells are stimulated, activation of the BLV provirus via NF-κB would result in increased transcription of viral proteins, including Tax, and increased viral replication. If BLV Tax acts in a manner similar to its HTLV-I counterpart, BLV Tax could, in turn, enhance binding of NF-κB to both cellular regulatory genes and the viral LTR. This positive cycle may explain the progression from long-term subclinical infection to a shorter period of preneoplastic polyclonal persistent lymphocytosis as selected subpopulations of lymphocytes are chronically stimulated. In addition, the viral Tax protein of HTLV-I has been shown to downregulate DNA repair (42). A similar function in BLV Tax would predispose rapidly dividing B cells to accumulate mutations, eventually resulting in a monoclonal lymphoma. The finding that BLV-infected B lymphocytes express high levels of IL-2Rα when stimulated (44) also suggests a multigene upregulation mediated by NF-κB. Further, the phenomena of spontaneous BLV-infected B-cell proliferation and viral transcription in vitro may involve NF-κB stimulation. Recent studies have shown that soluble Tax protein from HTLV is taken up by cells in culture and rapidly translocates to the nucleus, where it induces NF-κB binding activity (30).

As previously reported (3, 20), p65 did not appear to bind as a homodimer under the conditions in the footprint assay but did bind as a homodimer in EMSA. Ganchi et al. (17) have recently shown that p65 homodimers do occur in vivo and are independently regulated from the classic p50/p65 heterodimer combination. The nuclear extracts most likely have several species of NF-κB proteins which could form several homo- and heterodimer combinations. The binding sites seen in the DNase I protection assay may have different affinities for different κB heterodimers, and more than one may bind the NFLTR probe at once, which may account for the distinct shift with purified homodimers but complete aggregation of the probe with even low concentrations of several different types of nuclear extracts in EMSA. The BLV LTR κB site can compete specifically for binding, even at low molar ratios, with the κB consensus sequence. This may reflect multiple κB binding sites within the 53-bp fragment from the BLV LTR or differential binding affinities for the different κB heterodimers found in nuclear extracts. Preliminary studies with the 5′ half of this fragment suggest preferential binding for purified NF-κB p50, whereas the full fragment binds all three species of NF-κB in both footprinting assays and EMSA. Further studies are necessary to characterize the affinities and interactions of different NF-κB/Rel proteins on the BLV LTR.

Binding of CREB has been studied extensively on the BLV LTR and has recently been reported with a newly characterized protein, CREB2 (1, 45). Deletion mutant studies by Katoh et al. (26) and Derse (9) documented the importance of the three 21-bp repeats in BLV *trans*-activation. However, data from those studies suggested that the loss of the region encompassing the putative NF-κB binding site appeared to result in decreased enhancer activity independent of the loss of a CREB binding site. Numata et al. reported that HTLV-I has an NF-κB-like binding site between the second and third 21-bp repeats which acts synergistically with the third CRE (35). A similar relationship between these sites remains to be demonstrated for BLV.

Our finding that the BLV LTR contains a binding site for NF-κB proteins raises new implications for the relationship between transcriptional activation of the virus, activation of the host B cell, and neoplastic transformation of infected lymphocytes. It remains to be determined if NF-κB proteins are the common link between these events and to what extent they interact with the BLV *trans*-activating protein, Tax, and the other regulatory proteins such as CREB that bind to the LTR.

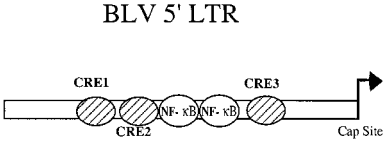

FIG. 7. Schematic map of protein-DNA interactions on BLV 5′ LTR.

ACKNOWLEDGMENTS

We thank Martine Uittenbogaard, Audrey Franklin, and Joel Rovnak for experimental expertise, helpful discussions, and technical advice.

This research was supported by Public Health Service grant RR00091-02 and USDA Food & Agricultural Sciences National Needs Graduate Fellowship 38420-3923 (to P.A.B.) and grants from the Research Council of the Colorado State University College of Veterinary Medicine and Biomedical Sciences (to G.L.C.).

REFERENCES

1. **Adam, E., P. Kerkhofs, M. Mammerickx, R. Kettmann, A. Burny, L. Droogmans, and L. Willems.** 1994. Involvement of the cyclic AMP-responsive element binding protein in bovine leukemia virus expression in vivo. J. Virol. **68:**5845–5853.
2. **Armstrong, A. P., A. A. Franklin, M. N. Uittenbogaard, H. A. Giebler, and J. K. Nyborg.** 1993. Pleiotropic effect of the human T-cell leukemia virus Tax protein on the DNA binding activity of eukaryotic transcription factors. Proc. Natl. Acad. Sci. USA **90:**7303–7307.
3. **Baeuerle, P. A.** 1991. The inducible transcription activator NF-κB: regulation by distinct protein subunits. Biochim. Biophys. Acta **1072:**63–80.
4. **Baeuerle, P. A., and T. Henkel.** 1994. Function and activation of NF-κB in the immune system. Annu. Rev. Immunol. **12:**141–179.
5. **Berkowitz, L. A., and M. Z. Gilman.** 1990. Two distinct forms of active transcription factor CREB (cAMP response element binding protein). Proc. Natl. Acad. Sci. USA **87:**5258–5262.
6. **Bours, V., G. Franzoso, K. Brown, S. Park, V. Azarenko, M. Tomita-Yamaguchi, K. Kelly, and U. Siebenlist.** 1992. Lymphocyte activation and the family of NF-κB transcription factor complexes. Curr. Top. Microbiol. **182:**411–420.
7. **Brogdon, W. G., and C. M. Dickinson.** 1983. A microassay system for measuring esterase activity and protein concentration in small samples and in high-pressure liquid chromatography eluate factions. Anal. Biochem. **96:**21–23.
8. **Cockerell, G. L., and J. Rovnak.** 1988. The correlation between the direct and indirect detection of bovine leukemia virus infection in cattle. Leuk. Res. **12:**465–469.
9. **Derse, D.** 1987. Bovine leukemia virus transcription is controlled by a virus-encoded *trans*-acting factor and by *cis*-acting response elements. J. Virol. **61:**2462–2471.
10. **Derse, D., and J. W. Casey.** 1986. Two elements in the bovine leukemia virus long terminal repeat that regulate gene expression. Science **231:**1437–1440.
11. **Duyao, M. P., D. J. Kessler, D. B. Spicer, and G. E. Sonenshein.** 1992. Transactivation of the c-*myc* gene by HTLV-1 *tax* is mediated by NFκB. Curr. Top. Microbiol. **182:**421–424.
12. **Dynan, W.** 1987. DNase I footprinting as an assay for mammalian gene regulatory proteins. Genet. Eng. **9:**75–87.
13. **Edbrooke, M. R., D. W. Burt, J. K. Cheshire, and P. Woo.** 1989. Identification of *cis*-acting sequences responsible for phorbol ester induction of human serum amyloid A gene expression via a nuclear factor κB-like transcription factor. Mol. Cell. Biol. **9:**1908–1916.
14. **Fischinger, P. J., C. S. Blevins, and S. Nomura.** 1974. Simple quantitative assay for both xenotropic murine leukemia and ecotropic feline leukemia viruses. J. Virol. **14:**177–179.
15. **Franklin, A. A., M. F. Kubik, M. N. Uittenbogaard, A. Brauweiler, P. Utaisincharoen, M.-A. Matthews, W. S. Dynan, J. P. Hoeffler, and J. K. Nyborg.** 1993. Transactivation by the human T-cell leukemia virus Tax protein is mediated through enhanced binding of activating transcription factor-2 (ATF-2) response and c-AMP element-binding protein (CREB). J. Biol. Chem. **268:**21225–21231.
16. **Gallo, R. C., and F. Wong-Staal.** 1982. Retroviruses as etiological agents of some animal and human leukemias and lymphomas and as tools for elucidating the molecular mechanism of leukemogenesis. Blood **60:**545–557.
17. **Ganchi, P. A., S.-C. Sun, W. C. Greene, and D. W. Ballard.** 1993. A novel NF-κB complex containing p65 homodimers: implications for transcriptional control at the level of subunit dimerization. Mol. Cell. Biol. **13:**7826–7835.
18. **Gorman, C. M., L. F. Moffat, and B. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
19. **Graves, D. C., and J. F. Ferrer.** 1976. *In vitro* transmission and propagation of the bovine leukemia virus in monolayer cell cultures. Cancer Res. **36:** 4152–4159.
20. **Grimm, S., and P. A. Baeuerle.** 1993. The inducible transcription factor NF-κB: structure-function relationship of its protein subunits. Biochem. J. **290:**297–308.
21. **Grueneberg, D. A., S. Natesan, C. Alexandre, and M. Z. Gilman.** 1992. Human and *Drosophila* homeodomain proteins that enhance the DNA-binding activity of serum response factor. Science **257:**1089–1095.
22. **Hoeffler, J. P., J. W. Lustbader, and C.-Y. Chen.** 1991. Identification of multiple nuclear factors that interact with cyclic adenosine 3′,5′-monophosphate response element-binding protein and activating transcription factor-2 by protein-protein interactions. Mol. Endocrinol. **5:**256–266.
23. **Hoeffler, J. P., T. E. Meyer, Y. Yun, J. L. Jameson, and J. F. Habener.** 1988. Cyclic AMP-responsive DNA-binding protein: structure based on a cloned placental cDNA. Science **242:**1430–1433.
24. **Jensen, W. A., J. Rovnak, and G. L. Cockerell.** 1991. In vivo transcription of the bovine leukemia virus *tax/rex* region in normal and neoplastic lymphocytes of cattle and sheep. J. Virol. **65:**2484–2490.
25. **Johnson, R., and J. B. Kaneene.** 1991. Bovine leukemia virus. Part I. Descriptive epidemiology, clinical manifestations, and diagnostic tests. Compend. Cont. Educ. Pract. Vet. **13:**315–326.
26. **Katoh, I., Y. Yoshinaka, and Y. Ikawa.** 1989. Bovine leukemia virus *trans*-activator $p38^{tax}$ activates heterologous promoters with a common sequence known as a cAMP-responsive element or the binding site of a cellular transcription factor ATF. EMBO J. **8:**497–503.
27. **Kettmann, R., Y. Cleuter, M. Mammerickx, M. Meunier-Rotival, G. Bernardi, A. Burny, and H. Chantreene.** 1980. Genomic integration of bovine leukemia virus: comparison of persistent lymphocytosis with lymph node tumor form of enzootic bovine leukosis. Proc. Natl. Acad. Sci. USA **77:**2577–2581.
28. **Kettmann, R., G. Marbaix, Y. Cleuter, D. Portetelle, M. Mammerickx, and A. Burny.** 1980. Genomic integration of bovine leukemia provirus and lack of viral RNA expression in the target cells of cattle with different responses to BLV infection. Leuk. Res. **4:**509–519.
29. **Lieberman, T. A., and D. Baltimore.** 1990. Activation of interleukin-6 gene expression through the NF-κB transcription factor. Mol. Cell. Biol. **10:**2327–2334.
30. **Lindholm, P. F., S. J. Marriott, S. D. Gitlin, C. A. Bohan, and J. N. Brady.** 1990. Induction of nuclear NF-κB DNA binding activity after exposure of lymphoid cells to soluble Tax_1 protein. New Biol. **2:**1034–1043.
31. **Matsuda, I., E. S. Masuda, A. Tsuboi, S. Behnam, N. Arai, and K. Arai.** 1994. Characterization of NF(P), the nuclear factor that interacts with the regulatory P sequence (5′-CGAAAATTTCC-3′) of the human interleukin-4 gene: relationship to NF-κB and NF-AT. Biochem. Biophys. Res. Commun. **199:**439–446.
32. **Mauxion, F., C. Jamieson, M. Yoshida, K.-I. Arai, and R. Sen.** 1991. Comparison of constitutive and inducible transcriptional enhancement mediated by κB-related sequences: modulation of activity in B cells by human T-cell leukemia virus type I *tax* gene. Proc. Natl. Acad. Sci. USA **88:**2141–2145.
33. **Müller, J. M., H. W. L. Ziegler-Heitbrock, and P. A. Baeuerle.** 1993. Nuclear factor kappa B, a mediator of lipopolysaccharide effects. Immunobiology **187:**233–256.
34. **Nabel, G., and D. Baltimore.** 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (London) **326:**711–713.
35. **Numata, N., K. Ohtani, M. Niki, and M. Nakamura.** 1991. Synergism between two distinct elements of the HTLV-I enhancer during activation by the trans-activator of HTLV-I. New Biol. **3:**896–906.
36. **Nyborg, J. K., W. S. Dynan, I. S. Y. Chen, and W. Wachsman.** 1988. Binding of host-cell factors to DNA sequences in the long terminal repeat of human T-cell leukemia virus type I: implications for viral gene expression. Proc. Natl. Acad. Sci. USA **85:**1457–1461.
37. **Nyborg, J. K., M. H. Matthews, J. Yucel, L. Walls, W. T. Golde, W. S. Dynan, and W. Wachsman.** 1990. Interaction of host cell proteins with the human T-cell leukemia virus type I transcriptional control region. J. Biol. Chem. **265:**8237–8242.
38. **Paul, P. S., K. A. Pomeroy, D. W. Johnson, C. C. Muscoplat, B. S. Handwerger, F. F. Soper, and D. K. Sorensen.** 1977. Evidence for the replication of bovine leukemia virus in the B lymphocytes. Am. J. Vet. Res. **38:**873–876.
39. **Pierce, J. W., M. Lenardo, and D. Baltimore.** 1988. Oligonucleotide that binds nuclear factor NF-κB acts as a lymphoid-specific and inducible enhancer element. Proc. Natl. Acad. Sci. USA **85:**1482–1486.
40. **Ruben, S. M., R. Narayanan, J. F. Klement, C.-Y. Chen, and C. A. Rosen.** 1992. Functional characterization of the NF-κB p65 transcriptional activator and an alternatively spliced derivative. Mol. Cell. Biol. **12:**444–454.
41. **Sagata, N., T. Yasunaga, J. Tsuzuku-Kawamura, K. Oshishi, and Y. Ogawa.** 1985. Complete nucleotide sequence of the genome of bovine leukemia virus: its evolutionary relationship to other retroviruses. Proc. Natl. Acad. Sci. USA **82:**677–681.
42. **Saggioro, D., F. Majone, M. Forino, L. Turchetto, A. Leszl, and L. Chieco-Bianchi.** 1994. Tax protein of human T-lymphotropic virus type I triggers DNA damage. Leuk. & Lymphoma **12:**281–286.
43. **Schmid, R. M., N. D. Perkins, C. S. Duckett, P. C. Andrews, and G. J. Nabel.** 1991. Cloning of an NF-κB subunit which stimulates HIV transcription in synergy with p65. Nature (London) **352:**733–736.
44. **Stone, D. M., T. F. McElwain, and W. C. Davis.** 1994. Enhanced B-lymphocyte expression of IL-2Rα associated with T lymphocytosis in BLV-infected persistently lymphocytotic cows. Leukemia **8:**1057–1061.
45. **Willems, L., R. Kettmann, G. Chen, D. Portetelle, A. Burny, and D. Derse.** 1992. A cyclic AMP-responsive DNA-binding protein (CREB2) is a cellular transactivator of the bovine leukemia virus long terminal repeat. J. Virol. **66:**766–772.
46. **Xie, Q., Y. Kashiwabara, and C. Nathan.** 1994. Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. J. Biol. Chem. **269:**4705–4708.