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Analysis of the Transcriptional Promoter Which Regulates the Latency-Related Transcript of Bovine Herpesvirus 1

Clinton J. Jones
University of Nebraska - Lincoln, cjones2@unl.edu

G. Delhon
University of Nebraska-Lincoln, gdelhon3@Unl.edu

A. Bratanich
University of Nebraska - Lincoln

G. Kutish
University of Nebraska - Lincoln

D. Rock
University of Nebraska-Lincoln, dlrock@illinois.edu

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Analysis of the Transcriptional Promoter Which Regulates the Latency-Related Transcript of Bovine Herpesvirus 1

C. JONES,[1]*† G. DELHON,[2] A. BRATANICH,[2] G. KUTISH,[2] AND D. ROCK[2]

Department of Microbiology, University of Mississippi Medical Center, Jackson, Mississippi 39216–4505,[1] and Department of Veterinary Science, University of Nebraska, Lincoln, Nebraska 68583[2]

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As a transcriptional promoter in primary cultures of sensory ganglionic neurons, DNA sequences near the 5′ terminus of the latency-related (LR) gene of bovine herpesvirus 1 were at least 10 times more efficient than the simian virus 40 early promoter-enhancer. In contrast, as a promoter in bovine, rodent, or monkey cells, the LR promoter was approximately six times less efficient than the simian virus 40 early promoter-enhancer. The LR promoter had strict orientation preferences in neurons and all other mammalian cell lines tested. Removal of a 146-base-pair *Xho*I fragment from the LR promoter resulted in stimulation of LR promoter activity in bovine cells but not rabbit neurons, monkey fibroblasts, or rodent cells. LR promoter activity in bovine cells is stimulated by bovine herpesvirus 1 lytic infection, suggesting that viral gene products or virus-induced factors positively regulate the expression of the LR gene. A synthetic glucocorticoid, dexamethasone, repressed LR promoter activity in bovine cells. These results imply that a variety of factors can influence the expression of the LR gene during latent infections of neurons as well as during the lytic infection cycle.

Bovine herpesvirus 1 (BHV-1), like other members of the alphaherpesvirus family, establishes latent infections in neurons (1, 2, 6, 15, 17, 18). Following a primary infection, the virus persists in the neurons of sensory and autonomic nerve ganglia of infected cattle (1, 6, 15). Spontaneous and sporadic reactivation of the virus occurs in latently infected animals, and interestingly, latent virus can be reactivated predictably from infected animals following the administration of glucocorticoids (2, 4, 7, 9, 17, 18). The nature of the virus-cell interaction that results in initiation, maintenance, and reactivation of this latent infection is unknown.

Investigations into the molecular basis of BHV-1 latency by means of a rabbit latency model (14, 15) have shown that the BHV-1 genome is transcriptionally active in latently infected ganglionic neurons, with transcription restricted to a region approximately 1.16 kilobases in size (0.740 to 0.748 map units) within *Hin*dIII fragment D (14; D. Rock, unpublished data). DNA sequence analysis of this region reveals two major open reading frames within the approximate map positions of the latency-related (LR) RNA and a potential Pol II promoter in close proximity to the 5′ terminus of the LR RNA (Rock, unpublished data). While it seems reasonable to hypothesize a role for this LR gene in viral latency or viral reactivation or both, the functional significance of its expression in latently infected neurons is unknown.

Given the probable significance of this gene in some aspect of BHV-1 latent infection, it undoubtedly will be important to understand the mechanisms governing its expression in sensory neurons. We examined the transcriptional promoter activity of the LR gene in a variety of cell types, including primary rabbit sensory neurons. The LR promoter was an efficient promoter in primary cultures of sensory ganglionic neurons compared with the simian virus 40 (SV40) early promoter-enhancer. In sharp contrast, the SV40 early promoter-enhancer was a better promoter in all other cells tested. Our results indicate that the LR promoter has strict orientation preferences in all cell types tested and that in bovine cells, promoter activity is stimulated by infection with BHV-1. A 146-base-pair (bp) *Xho*I fragment in the LR promoter functioned as a "silencer" in two different bovine cell lines. However, in monkey or rodent cells, the *Xho*I fragment enhanced promoter activity. A synthetic glucocorticoid, dexamethasone (DEX), regulated LR promoter activity in a negative fashion. Taken together, these results indicate that the LR gene of BHV-1 is regulated by tissue- and species-specific transcription factors as well as viral or virus-induced factors.

MATERIALS AND METHODS

Cells and viruses. Bovine lung and bovine turbinate cells were used throughout the experiments and maintained as described previously (16). The Cooper strain of BHV-1, obtained from the National Veterinary Services Laboratory, Animal and Plant Health Inspection Service, Ames, Iowa, was used at passage 10. In infection experiments, bovine cell cultures or primary rabbit sensory neurons were infected with BHV-1 at a multiplicity of infection (MOI) of 10 either 5 or 18 h prior to harvest. Cell lines CV-1 (monkey fibroblasts), Rat-2 (rat fibroblasts), and JB-6 (mouse epithelial cells) were maintained as described previously (8). The JB-6 cell line, C141, was obtained from Nancy Colburn (National Cancer Institute).

Nerve cell cultures. Sensory neurons from dorsal root ganglia were obtained from 1- to 3-day-old rabbits. Forty to fifty dorsal root ganglia were collected per animal and dissociated by treatment with 0.25% collagenase in Eagle minimal essential medium for 4 h at 37°C. The suspension was plated onto collagen-coated dishes at 1.7×10^4 neurons per well (1.8 cm^2) in growth medium (minimal essential medium with 1.4 mM glutamine, 25 μg of gentamicin per ml, 50 ng of nerve growth factor per ml, and 12 mg of glucose per ml) supplemented with 30% fetal calf serum and maintained

* Corresponding author.
† Present address: Department of Veterinary Science, University of Nebraska, Lincoln, NE 68583.

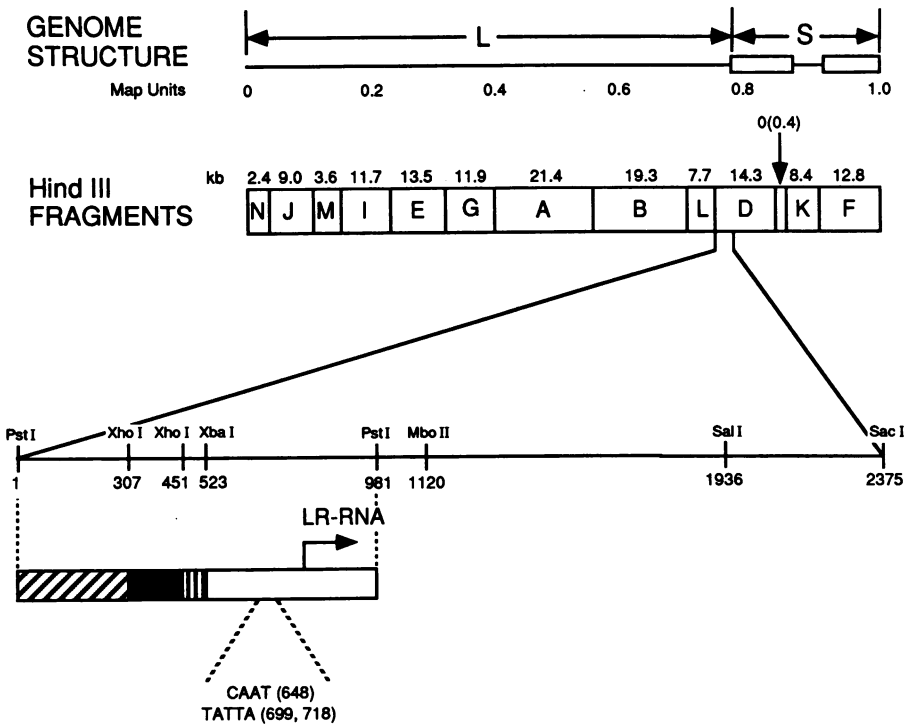


FIG. 1. BHV-1 DNA (Cooper strain). This linear double-stranded molecule of approximately 136 kilobases consists of two unique sequence regions, L and S, the smaller of which is bounded by inverted repeat sequences (12). The BHV-1 LR RNA maps to a region of approximately 1.16 kilobases at the left-hand side of *Hin*dIII fragment D. The approximate 5′ and 3′ ends of the LR RNA fall between nucleotides 773 to 981 and 1753 to 1936, respectively (Rock, unpublished data). The 981-bp *Pst*I fragment used in making the LR promoter-CAT constructs for these experiments (shown below the expanded restriction map of the LR region) contains the 5′ terminus of the LR RNA and additional upstream sequences. The direction of the LR RNA (5′ to 3′) is shown by the arrow. Putative CAAT and TATTA box regions for the LR RNA are indicated.

at 37°C with 5% CO_2. After 1 day in culture, the cells were treated for 10 days with 10 μM 5-fluorodeoxyuridine and 10 μM cytosine arabinoside in growth medium containing 10% fetal calf serum. This procedure removed contaminating nonneural cells. Following treatment, more than 95% of the cells were sensory neurons as judged from cell morphology. For all transfection experiments, 7×10^4 neurons (18 to 20 days old) per plasmid construct were used. Neuronal cell networks free from residual nonneural cells were removed from the collagen surface by vigorously pipetting the culture fluid within the well for analysis of chloramphenicol (CM) acetyl transferase (CAT) activity.

CAT assays. The measurement of CAT activity was described previously (8). Forty hours after transfection, cells were washed three times with phosphate-buffered saline and a cell lysate was prepared by three freeze-thaw cycles in 0.25 M Tris (pH 7.8). Cell debris was pelleted by centrifugation, and the protein concentration was measured (3). Amounts of protein used in the CAT assay are indicated in the figure legends: CAT activity was measured in a reaction mixture containing 0.2 μCi of [^{14}C]CM (45 mCi/mol), 100 mM Tris hydrochloride (pH 7.8), and 0.5 mM acetyl coenzyme A (5). After incubation for the indicated times (see figure legends) at 37°C, CM was extracted with ethyl acetate and the enzymatic products were analyzed by thin-layer chromatography (TLC) on silica gel in the solvent system $CHCl_3$-MeOH (95:5). TLC plates were autoradiographed at room temperature for 48 h. The amount of radioactivity associated with [^{14}C]CM and the various forms of acetylated [^{14}C]CM in TLC plates was measured with a System 400 imaging scanner (Bioscan).

Construction of BHV-CAT plasmids. The plasmid pSV2cat contains the SV40 early promoter and enhancer 5′ to the bacterial gene encoding CAT (5, 10). Plasmid pSV0cat is a promoter-enhancer-minus CAT plasmid which contains unique *Hin*dIII and *Bam*HI restriction enzyme sites 5′ and 3′, respectively, to the CAT gene. These plasmids were obtained from Bruce Howard (National Institutes of Health).

The 981-bp *Pst*I fragment containing the 5′ terminus of the LR gene (Fig. 1) was cloned into pUC18 such that nucleotide 1 of the viral fragment was proximal to the unique *Hin*dIII site in the polylinker of pUC18. The plasmid was linearized by digestion with *Sma*I, and subsequently synthetic *Hin*dIII linkers were added to the unique *Sma*I site in the polylinker region. Thus the 981-bp viral fragment could be released by *Hin*dIII digestion and contained 21 nucleotides of pUC18 polylinker from the *Sma*I site to nucleotide 981 of the viral *Pst*I fragment. The 981-bp BHV-1 fragment, the LR promoter region, was inserted at the 5′ terminus of pSV0cat (p0.95cat/1 and p0.95cat/6; Fig. 2). The orientations of p0.95cat/1 and p0.95cat/6 were deduced by *Sal*I-*Bam*HI digestion and *Sph*I-*Bam*HI digestion. Plasmid p0.95cat/1 was digested with *Xho*I to release the 146-bp *Xho*I fragment from the LR promoter, and the vector plus BHV-1 fragment was recircularized (pΔXho/1). Because of the presence of an *Xba*I site in the polylinker region of pUC-18, *Xba*I digestion of p0.95cat/1 released a 458-bp fragment of the LR promoter. This construct was subsequently designated p0.95ΔXba/4. After the addition of synthetic *Hin*dIII linkers, the *Xba*I-*Pst*I fragment of the LR promoter (nucleotides 523 to 981) was inserted into the unique *Hin*dIII site of pSV0cat in both orientations to produce p0.5Xba/47 and p0.5Xba/46. The

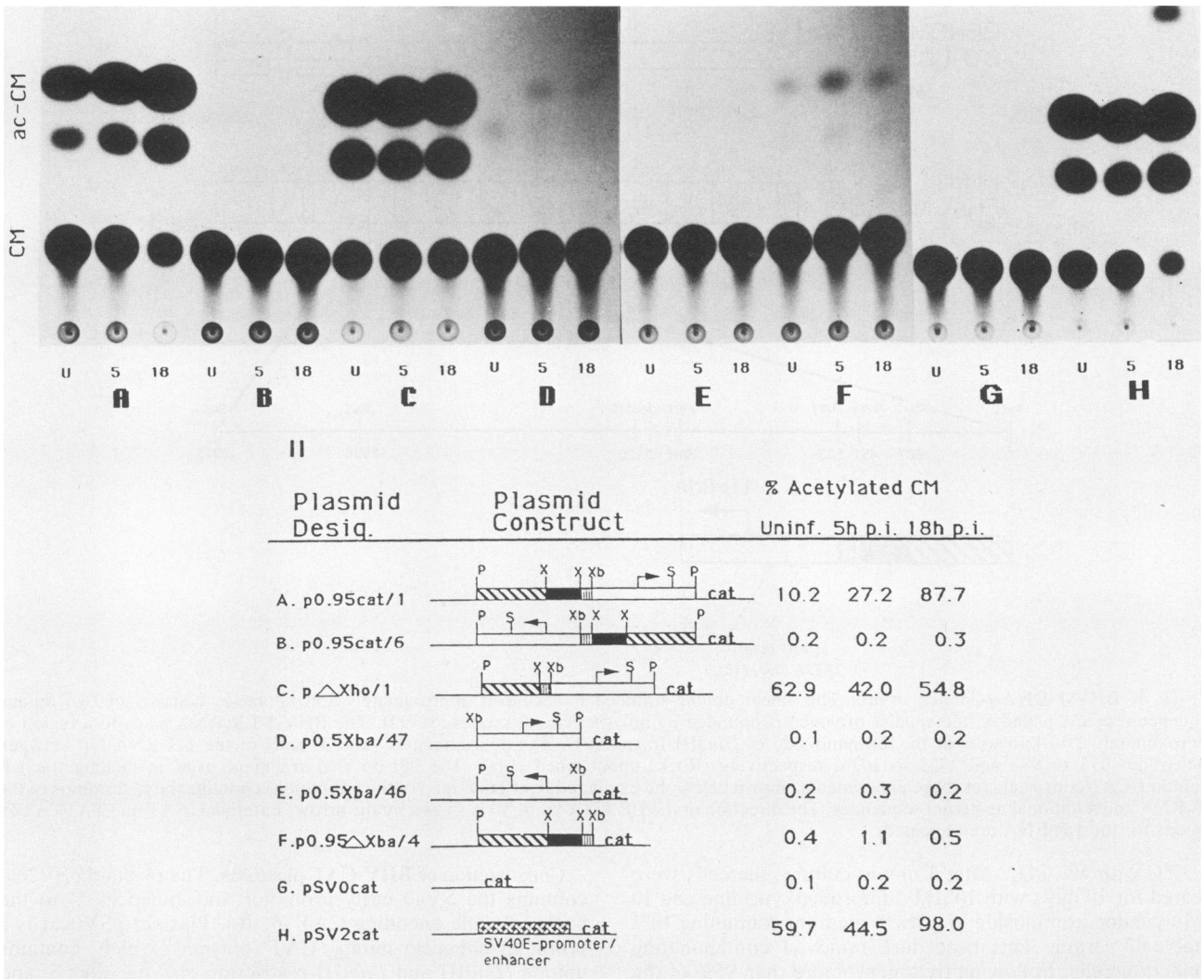


FIG. 2. Promoter activity of DNA fragments at 5′ terminus of LR gene. (I) Bovine cells were transfected with the indicated LR CAT plasmids. At 40 h posttransfection, cell lysates were prepared and subsequently assayed for CAT activity in the presence of [^{14}C]CM (40 μg of protein, 90-min assay at 37°C). Acetylated forms of CM (ac-CM) were separated from CM by TLC. Lanes U, Uninfected bovine cells; lanes 5, bovine cells infected for 5 h with BHV-1 (MOI, 10) prior to harvest of cells; lanes 18, bovine cells infected for 18 h with BHV-1 (MOI, 10) prior to the harvest of cells. (II) Designations of plasmid constructs used. The directions of the LR transcripts (5′ to 3′) and the approximate start sites are indicated by arrows. The direction of the CAT gene is from left to right. The levels of acetylated CM are expressed as percentages of the total radioactivity present in the various forms of CM. Restriction enzyme sites are P (*Pst*I), X (*Xho*I), S (*Sph*I), and Xb (*Xba*I). Uninf., Uninfected; p.i., postinfection.

orientations of p0.5Xba/47 and p0.5Xba/46 were deduced by *Sph*I-*Bam*HI digestion. The mouse mammary tumor virus long terminal repeat was obtained from plasmid pMAMneo (11). After the addition of synthetic *Hin*dIII linkers in the multiple cloning site of pMAMneo at the *Sma*I site, the mouse mammary tumor virus long terminal repeat was cloned into the unique *Hin*dIII site of pSV0cat in the proper orientation to produce pMMTVcat/4. The orientation of pMMTVcat was deduced by double digestion with *Sac*I-*Bam*HI.

RESULTS

***cis* activation of CAT gene by BHV-1 LR sequences.** To examine the transcriptional promoter activity of the LR gene, a 981-bp *Pst*I fragment (LR promoter) which contains the 5′ terminus of the LR transcript was inserted adjacent to a promoter-minus CAT plasmid, pSV0cat, and promoter activity was examined in two bovine cell lines. The two bovine cell lines used in these studies were bovine lung and bovine turbinate. For simplicity, we present the data obtained from bovine turbinate cells; however, identical results were obtained with bovine lung cells (data not shown). The LR promoter region was active only if oriented so the 5′ sequences of the LR transcript were adjacent to the CAT-coding sequences of p0.95cat/1 (Fig. 2). A plasmid, p0.95cat/6, which contains the 981-bp *Pst*I fragment in the opposite orientation had little or no promoter activity. Removal of a 146-bp *Xho*I fragment from the LR promoter, pΔXho/1,

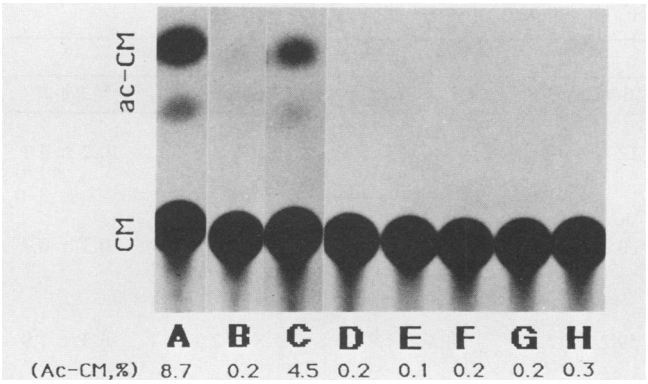


FIG. 3. LR promoter activity in ganglionic sensory neurons. Neurons derived from rabbit sensory ganglia were transfected with plasmids. At 40 h posttransfection, the cells were harvested and CAT enzymatic activities were measured in the presence of [^{14}C]CM (15 μg of protein, 2 h, 37°C). Lanes: A, p0.95cat/1; B, p0.95cat/6; C, pΔXho/1; D, p0.5Xba/47; E, p0.5Xba/46; F, p0.95ΔXba/4; G, pSV0cat; H, pSV2cat. The level of acetylated CM (ac-CM) is expressed as a percentage of the total radioactivity present in the various forms of CM.

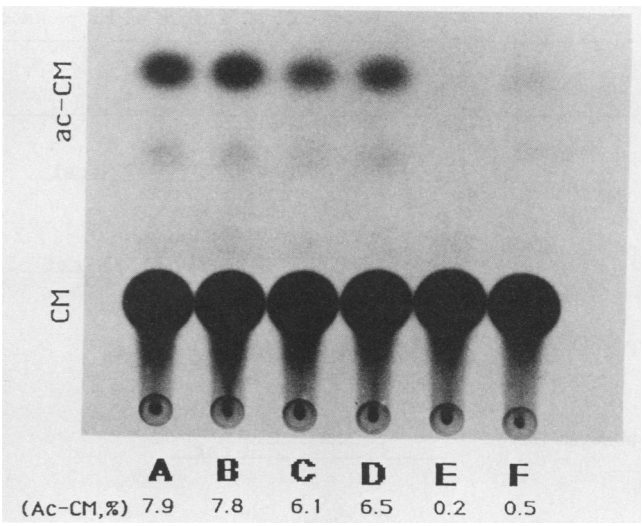


FIG. 4. LR promoter activity in ganglionic sensory neurons infected with BHV-1. Neurons were transfected with the designated plasmids (10 μg). At 40 h posttransfection, cells were harvested and CAT enzymatic activity was measured (15 μg of protein, 2 h, 37°C) as described in Materials and Methods. Lanes: A, p0.95cat/1; B, p0.95cat/1, infected for 18 h with BHV-1 (MOI, 10) prior to harvest of cells; C, pΔXho/1; D, pΔXho/1 infected for 18 h with BHV-1 (MOI, 10) prior to harvest; E, pSV2cat; F, pSV2cat infected for 18 h with BHV-1 (MOI, 10) prior to harvest. The level of acetylated CM (ac-CM) is expressed as a percentage of the total radioactivity present in the various forms of CM.

stimulated promoter activity at least sixfold with respect to p0.95cat/1. Furthermore, pΔXho/1 had levels of promoter activity equivalent to those of the SV40 early promoter-enhancer, pSV2cat. The individual *Xba*I fragments of the 981-bp fragment (p0.5Xba/47, p0.5Xba/46, and p0.95ΔXba/4) had only basal promoter activity in transfected bovine cells. As expected, the promoter-minus vector, pSVOcat, displayed no promoter activity in bovine cells. These results suggest that the LR promoter was an orientation-dependent transcriptional promoter which contained an element, the *Xho*I region, that functioned as a silencer in bovine cells. Furthermore, the *Xba*I site of the LR promoter spans a critical region, since the individual *Xba*I fragments were not efficient promoters in bovine cells.

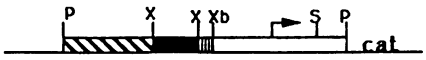
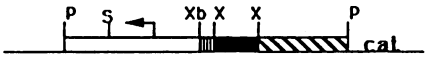
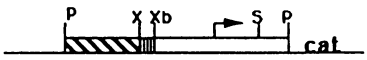
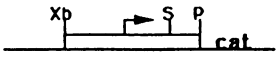
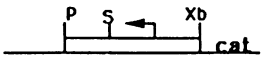
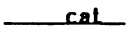
To assess the role lytic virus replication has on LR promoter activity, the various LR promoter constructs were transfected into bovine cells and subsequently infected with BHV-1 (MOI, 10) for 5 or 18 h prior to harvest. The promoter activity of p0.95cat/1 was clearly stimulated after viral infection (Fig. 2). Promoter activity was more than eightfold higher in cells infected for 18 h than in uninfected cells. The orientations of the LR promoter sequences were also critical for transactivation after BHV-1 infection, since p0.95cat/6 had no promoter activity in uninfected or infected bovine cells. The promoter activity of pΔXho/1 did not achieve the high level of p0.95cat/1 at 18 h postinfection. As expected, little if any promoter activity was detected in cells (normal or infected) transfected with a promoter-enhancer-minus CAT plasmid, pSV0cat. In three independent experiments, the activity of the SV40 early promoter-enhancer, pSV2cat, was increased slightly in bovine cells infected with BHV-1. In contrast, infection of monkey cells (CV-1) by human herpes simplex virus 2 reduced pSV2cat promoter activity at late times during the lytic infection cycle (C. Jones, unpublished data). These results indicate LR promoter activity was enhanced by viral infection, and they further imply that the *Xho*I region of the LR promoter plays a role in efficient transactivation after BHV-1 infection.

LR promoter activity in neurons. Since the LR transcript is the exclusive transcript in rabbit sensory neurons latently infected with BHV (14), the LR promoter should function efficiently in ganglionic sensory neurons. To assess LR promoter activity in cultured rabbit sensory neurons, cells were transfected with the various CAT constructs, and at 40 h posttransfection, levels of CAT activity were measured. The 981-bp *Pst*I fragment (p0.95cat/1) was >10 times more efficient as a promoter in neurons than was the SV40 early promoter-enhancer (pSV2cat; Fig. 3). As in bovine cells, the orientation of the 981-bp fragment (p0.95cat/6) was crucial for promoter activity. Removal of the *Xho*I region from the LR promoter (pΔXho/1) consistently had a slight negative effect on promoter activity. The individual *Xba*I fragments (p0.5Xba/47, p0.5Xba/46, and p0.95ΔXba/4) had no promoter activity in neurons. Taken together, the results indicate that in neurons, the LR promoter *cis* activated the CAT gene more efficiently than the SV40 early promoter-enhancer did. Furthermore, the *Xho*I region did not silence promoter activity in neurons as it did in bovine cells.

To test the effect viral infection had on promoter activity, neurons were transfected with the various promoter constructs and subsequently infected with BHV-1 18 h prior to measurement of CAT activity. The results indicate that infection had little effect on LR promoter activity (p0.95cat/1 and pΔXho/1; Fig. 4). Infection of neurons by BHV-1 also had little effect on the promoter activity of the individual *Xba*I fragments (p0.5Xba/47, p0.5Xba/46, and p0.95ΔXba/4; data not shown). Thus, in sharp contrast to results from bovine cells, LR promoter activity was not stimulated by the infection of neurons with BHV-1.

LR promoter activity in primate and rodent cells. Promoter activity of the various LR promoter constructs in other mammalian cell lines was measured to compare it with that observed in bovine cells. The results of these experiments in three cell lines (CV-1, Rat-2, and JB-6) are presented in Table 1. As in bovine cells, (i) p0.95cat/1 had substantial

TABLE 1. LR promoter activity in mammalian cells[a]

Plasmid designation	Plasmid construct[b]	Promoter activity[c] of acetylated CM (%) in:			
		Bovine cells	CV-1	JB-6	Rat-2
p0.95cat/1		12.4 ± 4.5	7.8 ± 3.2	9.5 ± 3.6	10.2 ± 4.1
p0.95cat/6		0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	0.3 ± 0.2
pΔXho/1		59.2 ± 12.3	4.4 ± 1.7	5.8 ± 2.3	6.3 ± 1.9
p0.5Xba/47		0.5 ± 0.3	0.9 ± 0.5	1.2 ± 0.7	0.5 ± 0.3
p0.5Xba/46		0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
p0.95ΔXba/4		0.5 ± 0.3	0.4 ± 0.2	0.7 ± 0.2	0.6 ± 0.3
pSV0cat		0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.2
pSV2cat		65.8 ± 15.7	58.4 ± 13.8	62.3 ± 17.6	68.4 ± 19.2

[a] Cells were transfected with the various plasmids (10 μg). At 40 h posttransfection, total-cell lysates were prepared and CAT activity was measured in the presence of [^{14}C]CM (40 μg of protein, 90 min, 37°C). The various forms of CM were separated by TLC and quantified.

[b] Restriction enzyme sites: P, *Pst*I; X, *Xho*I; S, *Sph*I; Xb, *Xba*I.

[c] Results are the averages of three independent experiments (bovine cells, CV-1, and Rat-2) or two experiments (JB-6) ± standard deviation.

promoter activity that was significantly lower than the promoter activity of pSV2cat; (ii) if the orientation of the BHV-1 insert was opposite that of p0.95cat/1 (p0.95cat/6), no promoter activity was observed; (iii) the individual *Xba*I fragments, p0.5Xba/47 and p0.95ΔXba/4, also had little or no promoter activity. In sharp contrast to the situation in bovine cells, removal of the 146-bp *Xho*I fragment had a slight but reproducible negative effect on promoter activity in the cell lines tested. These results indicate that the BHV-1 LR promoter functioned in a variety of nonbovine cell lines and also imply that a bovine-specific factor(s) regulated this viral promoter.

Effect of DEX on promoter activity. DEX treatment of animals latently infected with BHV-1 leads to reactivation of the virus and a transient decrease in the number of LR RNA-containing neurons present in ganglia (4, 9, 17; Rock, unpublished data). Thus, DEX may induce reactivation or reduce the expression of the LR gene. To test the effect DEX has on the LR promoter, bovine cells were transfected with the designated plasmids, and promoter activity in cells treated with DEX and in untreated cells was measured. DEX treatment consistently led to a reduction of promoter activity in cells transfected with p0.95cat/1 or pΔXho/1 (Fig. 5). Like Mordacq and Linzer (13), we observed that DEX treatment of mammalian cells decreased promoter activity of the SV40 early promoter-enhancer (data not shown). In sharp contrast to these results, the mouse mammary tumor virus long terminal repeat-CAT construct (pMMTV-4) was stimulated more than 10-fold by DEX treatment (Fig. 5; 11). DEX treatment of bovine cells downregulated the LR promoter and could thus conceivably play a role during the latent infection cycle.

DISCUSSION

We have identified and characterized the transcriptional promoter which regulates the expression of the LR transcript of BHV-1. The LR promoter contains a 146-bp element, the *Xho*I region, which silences promoter activity in bovine cells and seemingly prevents the efficient expression of the LR transcript until late in the lytic infection cycle. This same *Xho*I region seems to have a modest enhancing function in ganglionic sensory neurons, rodent cells, and monkey cells. In essence, the LR promoter is a complex yet diverse transcriptional promoter element which apparently is regulated by tissue-specific or species-specific transcription factors.

In bovine cells, the LR promoter was efficiently transactivated by BHV-1 infection (Fig. 2). Transactivation was clearly affected by the orientation of the LR promoter with respect to CAT-coding sequences. The sharp orientation preferences of the LR promoter were observed in all cell types tested as well. Removal of the *Xho*I region, yielding pΔXho/1, led to diminished promoter activity at 18 h post-

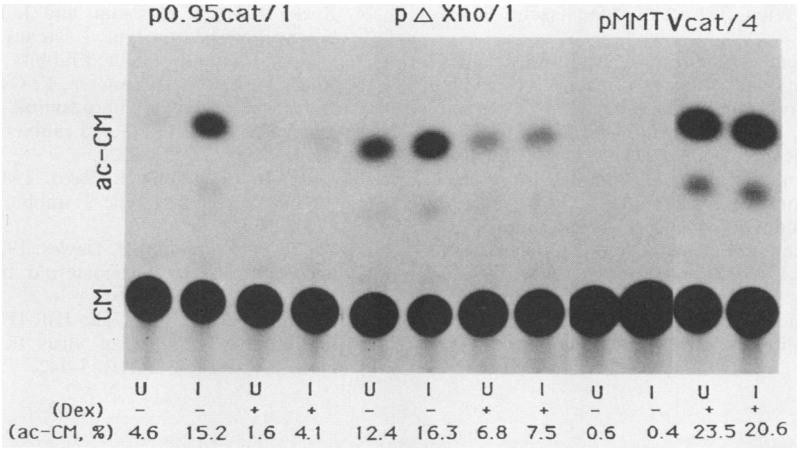


FIG. 5. Effect of DEX on LR promoter in bovine cells. Bovine cells were transfected with the designated plasmids (10 μg). DEX (10^{-6} M) was added to some dishes (+) after transfection but not to others (−). Cell lysates were prepared 40 h posttransfection. CAT enzymatic activity was measured as described in Materials and Methods (25 μg of protein, 1 h, 37°C). Lanes U, Normal uninfected bovine cells; lanes I, bovine cells infected with BHV-1 (MOI, 10) for 8 h prior to harvest of cells. The level of acetylated CM (ac-CM) is expressed as a percentage of the total radioactivity present in the various forms of CM.

infection when compared with p0.95cat/1. The *Xho*I region not only was involved with transactivation of promoter activity by BHV-1 infection but, more important, functioned as a negative regulatory element in bovine cells. This may imply that bovine-specific factors are involved with regulating LR promoter activity.

The LR promoter was an extremely efficient promoter in sensory ganglionic neurons when compared with the SV40 early promoter-enhancer (Fig. 3 and 4). The LR promoter had at least 10-times-higher promoter activity than pSV2cat did in neurons. In contrast, pSV2cat was six- to eightfold more active in bovine, rodent, or monkey cells (Fig. 2, Table 1). This implies that cellular factors present in neurons can efficiently activate the LR promoter. Another possibility, which cannot be ruled out, is that neurons possess factors which specifically block some promoters, such as the SV40 early promoter-enhancer. In contrast to the results observed in bovine cells, promoter activity in neurons was slightly repressed by removal of the *Xho*I region. This seems to support the notion that species- and tissue-specific factors regulate, in part, LR promoter activity. As in bovine cells, the individual *Xba*I fragments had little or no promoter activity. Quite surprisingly, infection of neurons by BHV-1 had little effect on LR promoter activity. Perhaps infection of cultured neurons was an inefficient process, and thus few cells were infected. However, these results clearly indicate that the LR promoter was an efficient promoter in neurons and thus explain, in part, the efficient expression of the LR transcript during latent infection of neurons by BHV-1. It should prove quite interesting to examine the interplay of cellular and viral factors with the LR promoter regulatory region and ultimately to determine how these factors regulate expression of the LR transcript.

The fact that a synthetic glucocorticoid, DEX, had a profound negative effect on LR promoter activity is particularly interesting given its ability to induce viral reactivation in latently infected animals (4, 9, 16, 17). This in vitro finding is in good agreement with studies that examined the early ganglionic events surrounding DEX-induced BHV-1 reactivation. Previous studies demonstrated that the number of neurons expressing LR RNA in latently infected ganglia decreased significantly (60%) 24 to 48 h posttreatment, with a return to control values by 72 h posttreatment. (Rock et al., unpublished data). Considering the negative effect DEX has on LR promoter activity, the transient decrease in LR RNA-containing ganglionic neurons is best explained as downregulation of LR RNA expression in these cells. The positive correlation of latent viral reactivation with this decrease makes it tempting to speculate that altered regulation of LR RNA transcription is a significant event in latent viral reactivation.

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LITERATURE CITED

1. **Ackerman, M., E. Peterhans, and R. Wyler.** 1982. DNA of bovine herpes virus type 1 in the trigeminal ganglia of latently infected calves. Am. J. Vet. Res. **43**:36–40.
2. **Bitsch, V.** 1973. Infectious bovine rhinotracheitis virus infection in bulls, with special reference to preputial infection. Appl. Microbiol. **26**:337–343.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–264.
4. **Davies, D. H., and L. E. Carmichael.** 1973. Role of cell-mediated immunity in the recovery of cattle from primary and recurrent infections with infectious bovine rhinotracheitis virus. Infect. Immun. **8**:510–518.
5. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
6. **Homan, E. J., and B. C. Easterday.** 1983. Experimental latent and recrudescent bovine herpesvirus-1 infections in calves. Am. J. Vet. Res. **44**:309–313.
7. **Huck, R. A., P. G. Miller, and D. G. Woods.** 1973. Experimental infection of maiden heifers by the vagina with infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus. J. Comp. Pathol. **83**:271–279.
8. **Jones, C.** 1989. The minimal transforming fragment of HSV-2 mtrIII can function as a complex promoter element. Virology **169**:346–353.
9. **Kubin, G.** 1969. Intermittent recovery of IPV virus from a

naturally infected bull. Wien. Tieraerztl. Monatsschr. **56**:336–337.

10. **Laimins, L. A., G. Khoury, C. Gorman, B. Howard, and P. Gruss.** 1982. Host specific activation of transcripts by tandem repeats from simian virus 40 and Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. USA **79**:6453–6457.
11. **Lee, F.** 1981. Glucocorticoids regulate expression of dihydrofolate reductase cDNA in mouse mammary tumour virus chimaeric plasmids. Nature (London) **294**:228–232.
12. **Mayfield, J. E., P. J. Good, H. J. VanOort, A. R. Campbell, and D. E. Reed.** 1983. Cloning and cleavage site mapping of DNA from bovine herpesvirus 1 (Cooper strain). J. Virol. **47**:259–264.
13. **Mordacq, J. C., and D. I. H. Linzer.** 1989. Co-localization of elements required for phorbol ester stimulation and glucocorticoid repression of proliferin gene expression. Genes Dev. **3**:760–769.
14. **Rock, D. L., S. L. Beam, and J. E. Mayfield.** 1987. Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits. J. Virol. **61**:3827–3831.
15. **Rock, D. L., W. Hagemoser, F. Osorio, and D. E. Reed.** 1986. Detection of bovine herpesvirus type 1 RNA in trigeminal ganglia of latently infected rabbits by *in situ* hybridization. J. Gen. Virol. **67**:2515–2520.
16. **Rock, D. L., and D. E. Reed.** 1982. Persistent infection with bovine herpesvirus type 1: rabbit model. Infect. Immun. **35**: 371–373.
17. **Sheffy, B. E., and D. H. Davies.** 1972. Reactivation of a bovine herpesvirus after corticosteroid treatment. Proc. Soc. Exp. Biol. Med. **140**:974–976.
18. **Snowdon, W. A.** 1965. The IBR-IPV: reaction to infection and intermittent recovery of virus from experimentally infected cattle. Aust. Vet. J. **41**:135–142.