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Construction of a Recombinant Bovine Leukemia Virus Vector for Analysis of Virus Infectivity

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A recombinant bovine leukemia virus (BLV) was constructed in which the X region was replaced with the bacterial neomycin resistance gene controlled by the simian virus 40 early promoter. This virus, termed BLV-SVNEO, is a self-packaging, activator-dependent retroviral vector. Introduction of the plasmid pBLV-SVNEO into mammalian cells resulted in constitutive expression of the *neo* gene, whereas the BLV structural genes, *gag*, *pol*, and *env*, were expressed only in the presence of the two regulatory proteins, Tax and Rex. The production and release of recombinant virus by cells transfected with pBLV-SVNEO were proportional to the number of G418-resistant colonies that developed after susceptible cells were exposed to the filtered culture medium. BLV-SVNEO was able to infect cell lines of human, bovine, canine, feline, and murine origin. BLV-producing cell lines were resistant to superinfection with BLV-SVNEO. This cell-virus system should facilitate molecular genetic studies of BLV and will provide a rapid, quantitative measure of BLV infectivity in a variety of cell types. These studies also demonstrate the feasibility of using activator-dependent retroviral vectors such as BLV-SVNEO to deliver foreign genes into cells and eventually animals.

Bovine leukemia virus (BLV) is a retrovirus associated with a disease complex of cattle termed enzootic bovine leukosis, which is characterized by a very extended disease course often involving persistent lymphocytosis and culminating in B-cell lymphoma (1, 6). In experimentally infected sheep, the disease progresses more rapidly and a high percentage of animals develop lymphoma of B-cell origin (5, 8, 17). BLV is not transcribed in lymphocytes or tumor cells in vivo (11, 12, 23); however, virus is transiently produced when lymphocytes from the infected host are cultured in vitro (4, 14, 16). Infection of various cell types with BLV in vitro has only rarely resulted in the establishment of cell lines that continuously produce substantial amounts of virus; in most cases, BLV production is low, and it often decreases over time (7, 24). Furthermore, there is no in vitro transformation system for BLV analogous to T-cell transformation by human T-cell leukemia virus type I (HTLV-I).

BLV, HTLV-I, and HTLV-II are genetically and biologically related and use similar strategies to regulate gene expression. The X region at the 3′ end of the genome encodes two regulatory proteins termed Tax and Rex (15, 18, 20, 21). The Tax protein acts in concert with *cis*-acting sequence elements in the long terminal repeats to regulate transcription initiation and is essential for virus expression (2, 10, 19, 25). The Rex protein acts posttranscriptionally to modulate the levels of structural protein mRNAs (3); it has not been demonstrated that its activity is essential for virus replication.

Molecular genetic studies of both BLV and HTLV-I have been hindered by the highly restricted expression of these viruses. Usually, infection and provirus formation are followed by relatively low virus production. In experiments discussed here, BLV expression in cells infected with BLV or transfected with a cloned provirus was examined. In both cases, BLV expression was limited but could be increased by supplying Tax in *trans*. To monitor the BLV life cycle more easily, a recombinant provirus that contains a selectable marker controlled by an independent promoter was constructed. This recombinant allows one to measure BLV infections without relying on virus-controlled gene expression.

Expression of BLV in infected and transfected cells. We have shown previously that transfection of permanent cell lines with the BLV provirus clone, pBLV913 (Fig. 1), results in low levels of viral transcription (3). These levels could be increased substantially when pBLV913 was introduced into cells with plasmids that actively produced the transcriptional activator, Tax. It appeared that BLV could not initiate or maintain a positive feedback cycle leading to abundant expression in these transfected cells. In an attempt to determine whether cells transfected with pBLV913 are able to produce infectious virus, the plasmid was transfected into the human cell line RD-4 in combination with a *tax-rex* expression vector and pBL-H2neo (a plasmid analogous to pSV2neo in which the neomycin resistance gene is controlled by the BLV long terminal repeat). This strategy ensures that *tax* and *rex* will be expressed in G418-resistant cells (2). G418-resistant colonies obtained were individually expanded, and BLV expression was examined by Northern (RNA) blotting of poly(A)$^+$ RNA and by reverse transcriptase assays of the culture medium. One such cell clone, RD-4.4(BLV913), produced BLV at significant levels, as judged by RNA blotting and reverse transcriptase activity (Fig. 2; Table 1). It appeared that the normally restricted expression of BLV could be partially overcome by supplying excess Tax in *trans*. BLV expression in the RD-4.4(BLV913) cells was approximately 50 times lower than it was in the productively infected fetal lamb kidney cell line FLK-BLV.

The limited expression of BLV seen in transfected cells was also observed in BLV infection experiments. BLV-containing culture supernatant from FLK-BLV cells was used to infect RD-4 and RD-N7 cells. The RD-N7 cell line, which constitutively expresses BLV *tax* and *rex* genes, was previously derived by transfection of RD-4 cells (2). Reverse transcriptase activity and viral RNA synthesis were monitored periodically after the cells were infected. BLV expres-

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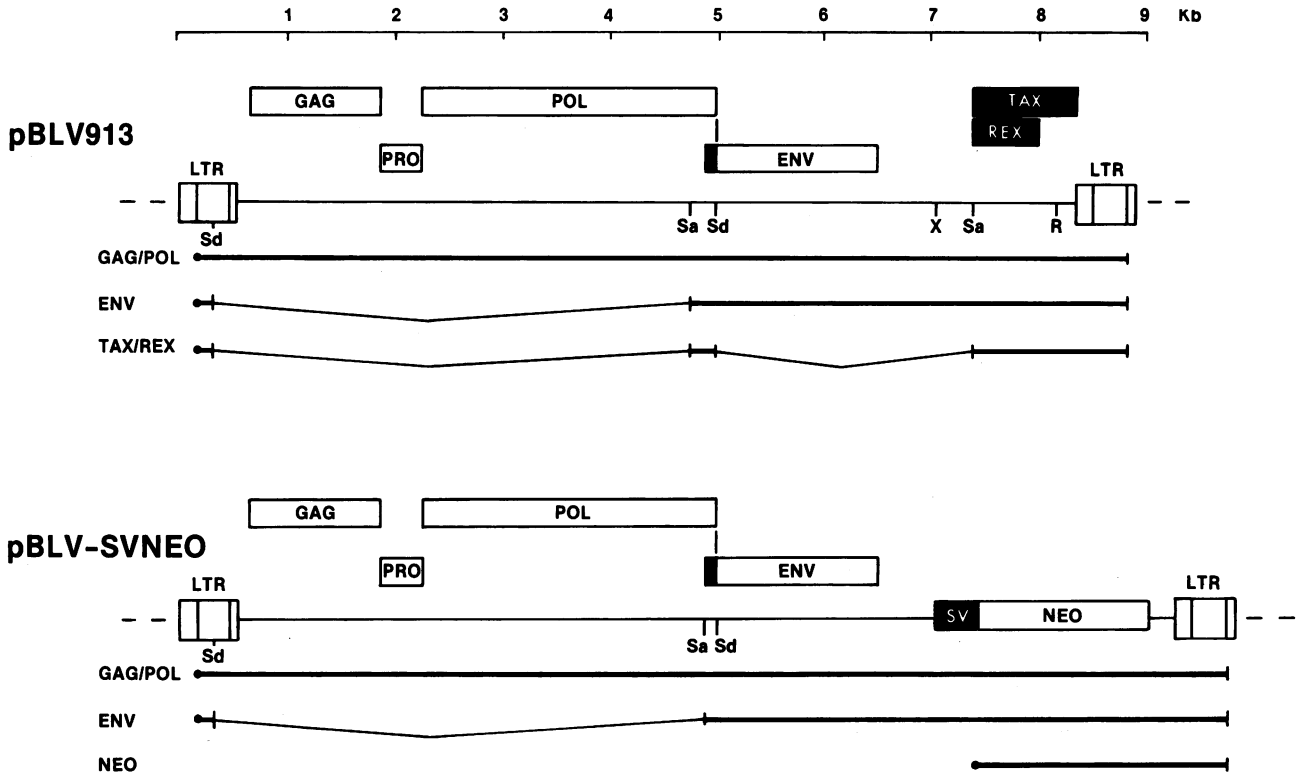

FIG. 1. Genetic organization and transcription pattern of pBLV913 and pBLV-SVNEO. The construction of pBLV913 has been previously described (3); it is a complete BLV provirus from which the *tax* and *rex* expression plasmids and pBLV-SVNEO were derived. Abbreviations: Sd, splice donor; Sa, splice acceptor; X, *Xho*I site; and R, *Eco*RI site. pBLV-SVNEO was made by replacing the 1,035-base-pair *Xho*I-to-*Eco*RI fragment, which contains the regulatory genes, with a cassette composed of the SV40 early promoter fused to the neomycin resistance gene, *neo*. Below each provirus are shown the respective mRNAs; for pBLV913, the full-length, singly spliced, and doubly spliced RNAs are translated to give *gag-pol*, *env*, and *tax-rex* proteins, respectively. pBLV-SVNEO does not yield a doubly spliced RNA, since it lacks the 3′ splice acceptor; a mRNA of approximately 2 kb is produced from the SV40 promoter and encodes the *neo* gene.

sion was negligible 2 weeks after infection of RD-4 cells (Fig. 2, lane 3; Table 1). In contrast, infection of RD-N7 cells resulted in significant virus expression (Fig. 2, lane 4; Table 1). Southern blot data revealed that the two cell cultures contained nearly equivalent levels of integrated BLV DNA (data not shown). Taken together and in light of earlier work, these data indicate that infections and transfections with BLV generally lead to the formation of provirus that is inefficiently expressed. Although the restricted expression can be overcome by artificially overproducing Tax, it is unclear why the provirus usually remains silent. These data also underscore the difficulty in examining the details of the virus life cycle; such analyses rely on the ability to monitor virus expression. The syncytium formation assay with certain fetal cell types permissive for BLV expression has been useful in examining the infectivity of cloned proviruses (9); however, this approach is limited by the cell types that can be used.

Construction of pBLV-SVNEO. It is clear that molecular genetic studies of BLV are very difficult to perform and reliably quantify because of the poor expression of the virus. Therefore, a selectable marker controlled by an independent promoter was inserted into BLV as a tag to monitor the virus. The 1,035-base-pair region at the 3′ end of the BLV genome, bounded by the *Xho*I and *Eco*RI sites, was replaced with a cassette composed of the simian virus 40 (SV40) early promoter fused to the bacterial neomycin resistance gene (Fig. 1). The recombinant provirus is designated BLV-SVNEO. It lacks the 3′ splice acceptor and coding sequences for the regulatory proteins Tax and Rex; however, the structural genes *gag*, *pol*, and *env* remain intact. Also shown in Fig. 1 is the predicted transcription pattern of the parent (pBLV913) and recombinant proviruses. Expression of the 2-kilobase (kb) *neo* mRNA is controlled by the SV40 early promoter, which is very active in most cell types and is independent of BLV regulation. BLV structural proteins Gag, Pol, and Env are translated from the full-length and singly spliced transcripts directed by the BLV long terminal repeat.

Transient expression of BLV-SVNEO in transfected cells. The patterns of *neo*-specific and BLV-specific mRNA synthesis were examined in D17 cells transfected with pBLV-SVNEO in combination with BLV *tax* and *rex* expression plasmids. At 2 days after transfection, poly(A)$^+$ RNA was prepared from the transfected cells and analyzed by Northern blotting with either *neo* or BLV *env* probes. The *neo*-specific RNA of approximately 2 kb, which is controlled by the SV40 promoter, was abundantly expressed in all transfected cultures (Fig. 3A). In contrast, accumulation of the full-length and singly spliced virus RNAs, initiated from the BLV long terminal repeat, was observed only in transfections in which BLV Tax was present (Fig. 3B). The addition of either BLV or HTLV-I Rex protein increased the amounts of genomic and singly spliced mRNAs. This is in agreement

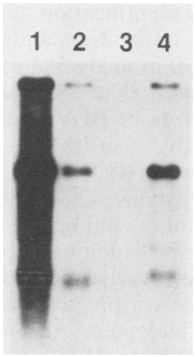

FIG. 2. Northern blot analysis of BLV RNA after transfection or infection of cells. Cytoplasmic poly(A)$^+$ RNA was purified by the hot acid phenol method and oligo(dT) cellulose chromatography as previously described (3). Poly(A)$^+$ RNA from 10^6 cells was loaded onto each lane of a 1% agarose–0.66 M formaldehyde gel, transferred to a nylon membrane, and hybridized with a BLV probe representing the 3′ end of the virus. The hybridizing bands represent 8.4-kb unspliced BLV genomic RNA, 4.1-kb singly spliced BLV RNA, and 1.7-kb doubly spliced RNA. RNAs from each of the following cell cultures were analyzed: FLK-BLV (lane 1), RD-4.4 (BLV913) (lane 2), BLV-infected RD-4 (lane 3), and BLV-infected RD-N7 (lane 4). RD-4.4(BLV913) is a clonal, G418-resistant cell line made by transfecting RD-4 cells with pBLV913, pBL-H2neo (a plasmid similar to pSV2neo that contains the BLV long terminal repeat), and pXB-RS (a plasmid that produces BLV Tax and Rex proteins) (2). RD-4 and RD-N7 cells were infected with filtered culture medium from FLK-BLV cells. Briefly, 10^6 cells were plated in 10-cm-diameter culture dishes; the next day, the medium was replaced with filtered medium from a semiconfluent flask of FLK-BLV cells. After 2 days, the medium was changed and the cells were passaged at weekly intervals. The RNAs shown here were prepared 2 weeks after the cells were infected. All cells were maintained in Dulbecco modified Eagle medium with 10% fetal calf serum.

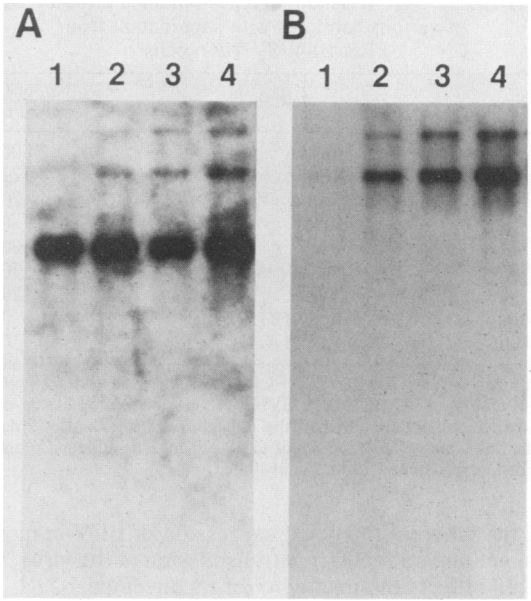

FIG. 3. Northern blot analysis of RNAs produced in cells transfected with pBLV-SVNEO. D17 cells were plated at a density of 10^6 cells per 10-cm-diameter culture dish the day before they were transfected. DNAs were applied as calcium phosphate coprecipitates. At 2 days after transfection, cells were collected and poly(A)$^+$ RNA was prepared and analyzed as described in the legend to Fig. 2. The blots were hybridized with a *neo* probe (A) and then stripped and rehybridized with a BLV *env* region probe (B). Each lane represents a transfection of cells with 10 μg of pBLV-SVNEO and 5 μg of plasmid cloning vector (lane 1), pBLV-tax (lane 2), pBLV-tax and pHTLV-rex (lane 3), or pBLV-tax and pBLV-rex (lane 4). The construction of the *tax* and *rex* expression plasmids will be described in detail elsewhere. Briefly, they contain cDNA copies of the *tax* or *rex* coding regions in plasmids that supply a Rous sarcoma virus promoter and SV40-poly(A) addition sequences.

with previous studies that showed that efficient synthesis of these RNAs requires both Tax and Rex (3) and that there is a cross-complementation of BLV and HTLV-I Rex proteins (D. Derse, unpublished observation). These results suggest that cells infected with BLV-SVNEO would produce *neo*-specific but not BLV-specific proteins.

The production and release of infectious virions from D17 cells transfected with both pBLV-SVNEO and *tax* and *rex* expression plasmids were examined next. The medium from each transfected culture used for the RNA analyses described above was filtered and transferred to dishes containing 5×10^5 HeLa cells; 2 days later, the medium was changed and G418 was added. Approximately 10 days after the start of growth in selective media, G418-resistant colonies could be scored; Table 2 summarizes the results from these experiments. This pBLV-SVNEO infectivity system revealed that both Tax and Rex were required for efficient production of infectious virus and that this recombinant virus is otherwise replication competent. The titers of virus produced in these experiments were quite low, perhaps because of a combination of problems, including the use of transient transfections rather than stably producing cell

TABLE 1. Reverse transcriptase activity in BLV-infected and transfected cells[a]

Cell type	RT activity (cpm)
FLK-BLV	3.0×10^5
RD-4	0.5×10^3
RD-4.4(BLV913)	5.1×10^3
RD-4 (BLV infected)	1.1×10^3
RD-N7 (BLV infected)	10.2×10^3

[a] At the time of cell harvesting for the RNA preparations described in the legend to Fig. 2, the culture media were removed and assayed for reverse transcriptase (RT) activity. The supernatant was clarified by high-speed centrifugation, and virus was pelleted by ultracentrifugation and assayed for reverse transcriptase activity as previously described (13). The data are expressed as counts per minute (cpm) of [^{3}H]thymidine incorporated into acid-precipitable material. The data represent the means of three separate determinations in which each assay was run in duplicate.

TABLE 2. Transfection of D17 cells with pBLV-SVNEO[a]

Cotransfected plasmids	No. of G418^r colonies
Null vector	0
pBLV-tax	2
pBLV-rex	0
pBLV-tax + pBLV-rex	50
pBLV-tax + pHTLV-rex	48

[a] D17 cells (10^6 cells per 10-cm-diameter dish) were transfected with 10 μg of pBLV-SVNEO in combination with 5 μg of the indicated expression plasmids as calcium phosphate coprecipitates as described in the legend to Fig. 3. After 2 days, the medium (10 ml) was collected, filtered, and transferred to cultures of 5×10^5 HeLa cells. At 2 days after infection, the medium was changed and the HeLa cells were maintained in medium containing 800 μg of G418 per ml. G418-resistant colonies were counted 10 days later.

TABLE 3. G418-resistant colonies obtained after infection of various cell lines with supernatant from FLK(BLV-SVNEO) cells[a]

Cell line	Source	No. of G418-resistant colonies
HeLa	Human	600
RD-4	Human	72
NIH 3T3	Mouse	20
LTK^-	Mouse	0
MDBK	Cow	300
D17	Dog	70
FEA	Cat	20
FLK-BLV	Sheep (BLV^+)	0
(BLV-bat	Bat (BLV^+)	0

[a] Culture medium (1 ml) from FLK-BLV cells stably transfected with pBLV-SVNEO (FLK-BLV/BLV-SVNEO) was added to 10-cm-diameter dishes containing 5×10^5 cells of the indicated cell lines. After 2 days, the medium was changed and 800 μg of G418 per ml was added. Colonies that developed were counted 10 to 15 days later.

lines, the inherent low-level expression of BLV in most cell types, and the poor cell-free transmission of the virus, which possibly reflects the instability of its envelope.

Susceptibility of various cell lines to BLV infection. Infection studies were performed by using a slightly different approach that yielded a higher-titer source of BLV-SVNEO. The FLK-BLV cell line (24), in which the viral structural and regulatory proteins are produced at very high levels, was transfected with pBLV-SVNEO. At 2 days after transfection, these cells were placed on G418 selection medium and the G418-resistant colonies that subsequently developed were pooled and expanded. Culture medium from this population of cells, termed FLK(BLV-SVNEO), should continuously produce both BLV and BLV-SVNEO; the cell line provides both regulatory and structural proteins to activate and package BLV-SVNEO. These cells released high levels of BLV-SVNEO into the medium, as was evidenced by the large number of G418-resistant colonies obtained in recipient cell lines (Table 3). The titers here were approximately 100 times higher than those from transiently transfected cultures. We have not yet characterized the proviruses in cells infected with virus from FLK(BLV-SVNEO) cultures to determine whether virus recombination has occurred. It is unlikely that recombination would generate a replication-competent virus that contains the SV-NEO fragment and the *tax* and *rex* genes.

In an attempt to determine what cell types would be infected by BLV and could serve as appropriate indicators of virus production, cells of human (HeLa), canine (D17), bovine (MDBK), feline (FEA), or murine (NIH 3T3 or LTK^-) origin were tested. The results show that most of these cell lines were susceptible to BLV infection (Table 3); HeLa cells were the most sensitive indicators (i.e., gave the most colonies), whereas the murine cell line, LTK^-, was the least infectible. The BLV-producing FLK cell line, FLK-BLV, and the bat lung cell line, BLV-bat, were resistant to superinfection with BLV-SVNEO, which suggests that this system reflects a specific, receptor-mediated infection. The broad range of cells infected with this virus mixture parallels that reported previously for BLV (7).

The utility of the pBLV-SVNEO system is demonstrated by the analysis of regulatory gene function presented above; both Tax and Rex proteins were shown to be required for production and release of virus particles. This system should also prove useful in further molecular genetic studies of BLV. For example, site-directed mutagenesis or deletion mutagenesis may allow identification of *cis*-acting sequences involved in replication, integration, and packaging. A similar approach will be helpful in analyzing functional domains in *gag*, *pol*, and *env* genes. The FLK(BLV-SVNEO) cells, which produce high titers of BLV and BLV-SVNEO, will facilitate in vitro analysis of the BLV host range and identification of a putative BLV receptor. This system has the advantage that almost any kind of recipient cell type can be examined. Furthermore, unlike the vesicular stomatitis virus pseudotype system, which has been used to show the existence of other retrovirus receptors (22), the BLV-SVNEO approach allows a positive selection of cells that express the receptor molecule. Although the preliminary screen of cells susceptible to BLV infection presented here is not exhaustive, it does corroborate previous studies that show a broad range of infectible cells and suggests that some cells, like mouse L cells, may be poor hosts for BLV. The easily quantified measure of BLV infectivity in a variety of cell types may be advantageous in studies of antiviral agents and virus neutralization by antisera.

The experiments with pBLV-SVNEO also demonstrate the potential utility of self-packaging, activator-dependent retroviral vectors as gene delivery systems. Since expression of viral proteins requires two activators, *tax* and *rex*, the recombinant virus is very much like a suicide vector; it is inert in infected cells lacking the regulatory proteins. Moreover, the recombinant virus lacks the genes *tax* and *rex*, which may be involved in leukemogenesis. This system may be of value for gene delivery to cells and eventually to animals.

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LITERATURE CITED

1. **Burny, A., C. Bruck, H. Chantrenne, Y. Cleuter, D. Dekegal, J. Ghysdael, R. Kettmann, M. Leclerq, J. Leunen, M. Mammerickx, and D. Portetelle.** 1980. Bovine leukemia virus: molecular biology and epidemiology, p. 231–289. *In* G. Klein (ed.), Viral oncology. Raven Press, New York.
2. **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.
3. **Derse, D.** 1988. *trans*-Acting regulation of bovine leukemia virus mRNA processing. J. Virol. **62**:1115–1119.
4. **Djilali, S., A.-L. Parodi, and D. Levy.** 1987. Bovine leukemia virus replicates in sheep B lymphocytes under a T cell released factor. Eur. J. Cancer Clin. Oncol. **23**:81–85.
5. **Djilali, S., A.-L. Parodi, D. Levy, and G. L. Cockerell.** 1987. Development of leukemia and lymphosarcoma by bovine leukemia virus in sheep: a hematopathological study. Leukemia **1**:777–781.
6. **Ghysdael, J., C. Bruck, R. Kettmann, and A. Burny.** 1984. Bovine leukemia virus. Curr. Top. Microbiol. Immunol. **112**: 1–19.
7. **Graves, D. C., and J. F. Ferrer.** 1976. In vitro transmission and propagation of bovine leukemia virus in monolayer cell cultures. Cancer Res. **36**:4152–4159.
8. **Hoss, H. E., and C. Olson.** 1974. Infectivity of bovine C-type (leukemia) virus for sheep and goats. Am. J. Vet. Res. **35**: 633–637.
9. **Itohara, S., and K. Sekikawa.** 1987. Molecular cloning of infectious proviral genomes of bovine leukemia virus. Virology **159**:158–160.
10. **Katoh, I., Y. Yoshinaka, N. Sagata, and Y. Ikawa.** 1987. The bovine leukemia virus X region encodes a trans-activator of its long terminal repeat. Jpn. J. Cancer Res. (Gann) **78**:93–98.
11. **Kettmann, R., J. Deschamps, Y. Cleuter, D. Couez, A. Burny,**

and G. Marbaix. 1982. Leukemogenesis by bovine leukemia virus: proviral DNA integration and lack of RNA expression of viral long terminal repeat and 3′ proximate cellular sequences. Proc. Natl. Acad. Sci. USA **79**:2465–2469.

12. **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.
13. **Klement, V., and M. O. Nicolson.** 1977. Methods for assays of RNA tumor viruses. Methods Virol. **6**:97–101.
14. **Levy, D., R. Kettmann, P. Marchand, S. Djilali, and A.-L. Parodi.** 1987. Selective tropism of bovine leukemia virus (BLV) for surface immunoglobulin-bearing ovine B lymphocytes. Leukemia **1**:463–465.
15. **Mamoun, R. Z., T. Astier-Gin, R. Kettmann, J. Deschamps, N. Rebeyrotte, and B. J. Guillimain.** 1985. The pX region of the bovine leukemia virus is transcribed as a 2.1-kilobase mRNA. J. Virol. **54**:625–629.
16. **Notzel, U., B. Drescher, and S. Rosenthal.** 1982. Detection of bovine leukemia virus RNA sequences in non-cultivated peripheral lymphocytes by in situ hybridization with ^{3}H-labelled cDNA. Acta Virol. **26**:33–40.
17. **Paul, P. S., K. A. Pomeroy, C. C. Muscoplat, B. S. Haudwergen, F. F. Soper, and D. K. Sorensen.** 1977. Evidence for replication of bovine leukemia virus in the B lymphocytes. Am. J. Vet. Res. **38**:873–876.
18. **Rice, N. R., S. L. Simek, G. C. Dubois, S. D. Showalter, R. V. Gilden, and R. M. Stephens.** 1987. Expression of bovine leukemia virus X region in virus-infected cells. J. Virol. **61**:1577–1585.
19. **Rosen, C. A., J. G. Sodroski, L. Willems, R. Kettmann, K. Campbell, R. Zaya, A. Burny, and W. A. Haseltine.** 1986. The 3′ region of bovine leukemia virus genome encodes a trans-activator protein. EMBO J. **5**:2585–2589.
20. **Sagata, N., J. Tsuzuku-Kawamura, M. Nagayoshi-Aida, F. Shimizu, K. Imagawa, and Y. Ikawa.** 1985. Identification and some biochemical properties of the major XBL gene product of bovine leukemia virus. Proc. Natl. Acad. Sci. USA **82**:7879–7883.
21. **Sagata, N., T. Yasunaga, and Y. Ikawa.** 1985. Two distinct polypeptides may be translated from a single spliced mRNA of the X genes of human T-cell leukemia and bovine leukemia viruses. FEBS Lett. **192**:37–42.
22. **Sommerfelt, M. A., B. P. Williams, P. R. Clapham, E. Solomon, P. N. Goodfellow, and R. A. Weiss.** 1988. Human T cell leukemia viruses use a receptor determined by human chromosome 17. Science **242**:1557–1559.
23. **Van den Broeke, A., Y. Cleuter, G. Chen, D. Portetelle, M. Mammerickx, D. Zagury, M. Fouchard, L. Coulombel, R. Kettmann, and A. Burney.** 1988. Even transcriptionally competent proviruses are silent in bovine leukemia virus-induced sheep tumor cells. Proc. Natl. Acad. Sci. USA **85**:9263–9267.
24. **Van Der Maaten, M. J., and J. M. Miller.** 1976. Replication of bovine leukemia virus in monolayer cell cultures. Bibl. Haematol. **43**:360–362.
25. **Willems, L., A. Gegonne, G. Chen, A. Burny, R. Kettmann, and J. Ghysdael.** 1987. The bovine leukemia virus p34 is a transactivator protein. EMBO J. **6**:3385–3389.