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Lack of Expression of Cellular Homologues of Retroviral *onc* Genes in Bovine Tumors*

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A. Introduction

Bovine leukemia virus (BLV), an exogenous retrovirus of cattle [5, 11], induces B-lymphocyte neoplasms (termed enzootic bovine leukosis, EBL) after long latent periods [2]. BLV contains no host cellular sequences and does not appear so far to bear genes capable of inducing transformation directly. A wide range of genomic sites can accomodate BLV proviruses. Transcription of viral DNA including long terminal repeated sequences has not been detected, strongly suggesting that viral gene expression is not required for maintenance of the tumor state. No expression of 3′-proximate cellular sequences has been observed, indicating that proximate downstream promotion did not take place in the cases examined [12].

The transforming genes of retroviruses are derived from normal cellular genes (*c-onc*) conserved among vertebrates [6, 17]. There is good evidence that virus-induced transformation is correlated with enhanced levels of expression of these genes [3, 14, 15]. Using labeled molecularly cloned DNA probes containing viral *onc* sequences, expression of cellular homologues of retroviral *onc* genes has been found in human tumor cells [7, 19]. Using the same approach, we examined whether or not one of these known *onc* genes was expressed at high level during the maintenance of the tumor state in bovine lymphosarcoma.

B. Results and Discussion

The viral *onc* genes used in the present investigation are listed in Table 1. Nick-translated DNA probes of each viral *onc* gene were first analyzed for their ability to detect homologous sequences in bovine DNA. Normal bovine cellular DNA was cleaved with *Eco*RI. The DNA fragments were subjected to agarose gel electrophoresis and to Southern blotting analysis in relaxed hybridization conditions [19]. As shown in Fig. 1 (lane 1), the *abl* DNA probe detected five DNA fragments of 6.2, 3.4, 2.8, 1.8, and 1.2 kb. The signal intensity was highest with the *abl* DNA probe, probably reflecting its greater homology with bovine DNA as compared to that of other viral *onc* genes tested. The other *onc* DNA probes (myc, erb, myb, src, ras, fes, and sis; Table 1) detected one or at most a few DNA fragments (data not shown). DNA from EBL tumors were analyzed in parallel (Fig. 1, lanes 2–7). For each *onc* probe tested, the patterns obtained were identical to the one observed with normal bovine DNA. These results indicate that in none of the EBL DNAs was there obvious rearrangement of any *onc* genes due to, for example, integration of the BLV provirus. The approach used to detect *onc*-related transcripts in bovine tumors involved isolation of poly (A)-containing RNAs and analysis by dot blot hybridization on nitrocellulose filters in relaxed hybridization

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Table 1. The different viral *onc* probes

Species	Virus strain	*onc* sequence	Viral clone obtained from
Avian	Avian myeloblastosis (MC 29)	myc	Dr. T. Papas [13]
	Avian erythroblastosis (AEV)	erb	Dr. M. Bishop [18]
	Avian myeloblastosis (AMV)	myb	Dr. M. Baluda [1]
	Rous sarcoma (RSV)	src	Dr. M. Bishop [4]
Murine	Abelson murine leukemia (Ab-MuLV)	abl	Dr. S. Aaronson [16]
	Harvey murine leukemia (Ha-MuSV)	ras	Dr. M. Martin [10]
Feline	Snyder-Theilen feline sarcoma (ST-FeSV)	fes	Dr. C. Scherr [8]
Simian	Simian sarcoma (SSV)	sis	Dr. R.C. Gallo [9]

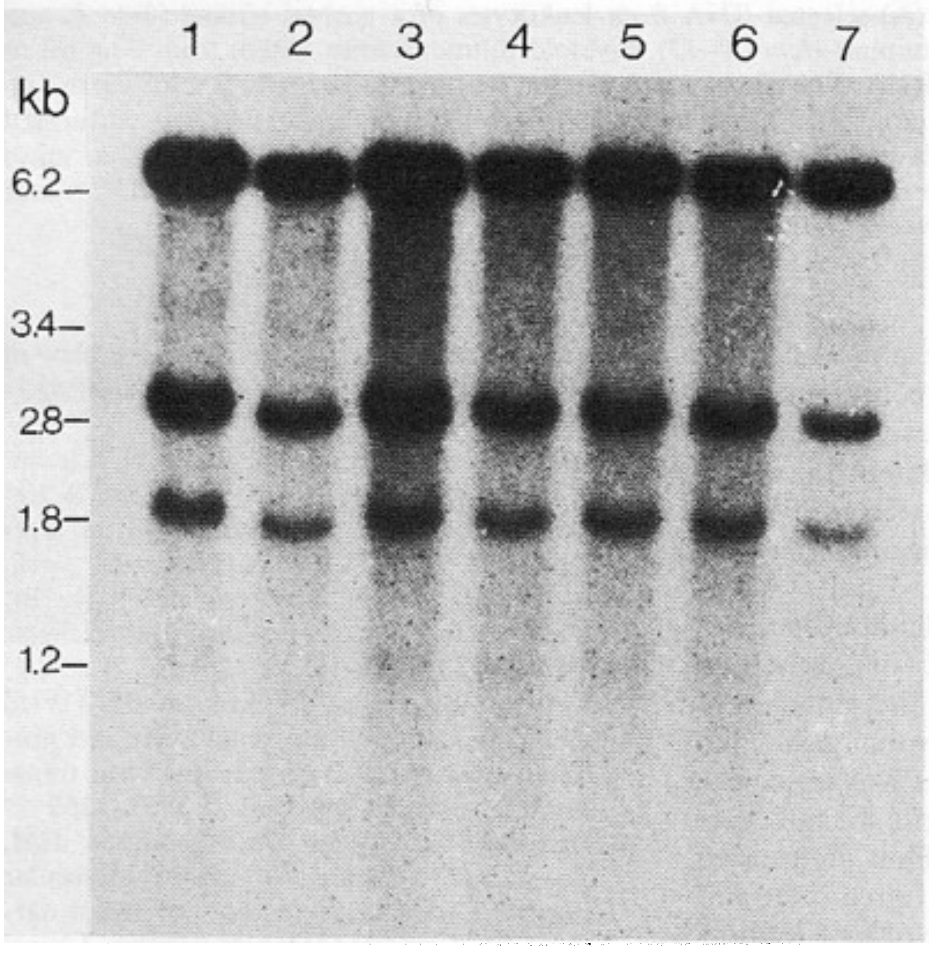

Fig. 1. Detection of bovine DNA sequences related to the *abl* retroviral *onc* gene. High-molecular weight DNA (10 μg) from normal bovine leukocytes (*lane 1*) and from EBL tumors (*lanes 2–7*) was digested with *Eco*RI and electrophoresed on an 0.8% agarose gel. Southern blot was prepared and incubated for 24 h with 2×10^6 c.p.m./ml nick-translated (2×10^8 c.p.m./μg) viral *onc* probe prepared from cloned DNA from Abelson MuLV. Hybridization was performed for 16 h at 37 °C in 50% formamide and 3×SSC with 10% dextran sulfate and followed by washings at 60°C with 2×SSC. The blot was exposed for 1 week using Kodak XAR-5 film and Dupont Cronex Lightning Plus screens. *Eco*RI generated fragments of bacteriophage λ DNA were used as molecular weight standards. *kb*, kilobase pairs

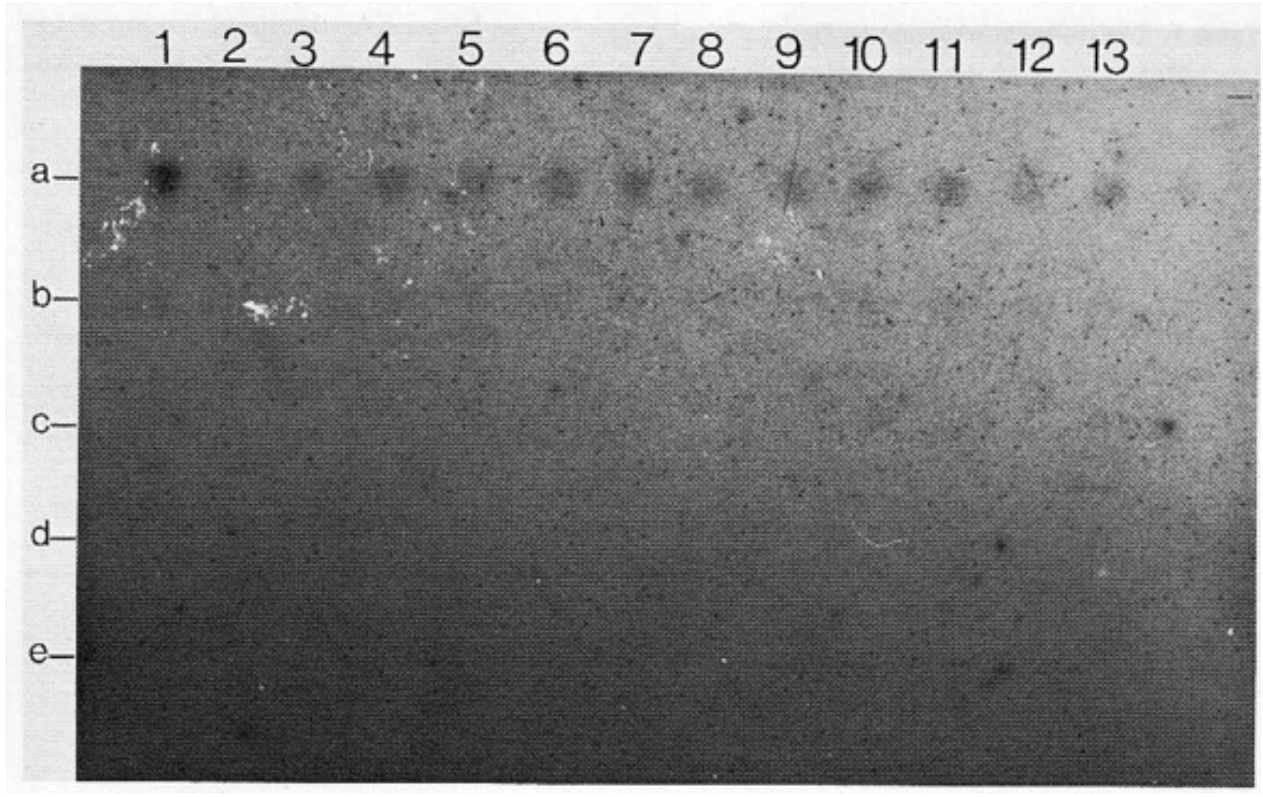

Fig. 2. Dot blot assay of poly (A)-selected RNA from leukocytes of a normal animal (*lane 1*) and from tumors of several other animals (*lanes 2–13*). Fivefold dilutions were tested, from 2 μg (*a*) to 3.2 ng (*e*) of poly (A)-selected RNA. The dot blot preparation was hybridized with 2×10^6 c.p.m./ml nick-translated (2×10^8 c.p.m./μg) cloned DNA from Abelson MuLV. Hybridization was performed in 50% formamide, 5×SSC with 10% dextran sulfate at 37 °C for 16 h. Filters were washed four times in 2×SSC/0.1% SDS at room temperature for 5 min each and then in 1×SSC/0.1% SDS at 42 °C for 15 min each. Autoradiography was a 1-week exposure

conditions. This technique allowed us to detect as little as 1 pg of complementary RNA. Bovine tumor cells were tested for the presence of viral *onc*-related transcripts both as total and poly (A)-selected RNAs. Figure 2 shows the hybridization between ^{32}P *abl* DNA as a probe and poly (A)-selected RNAs from normal leukocytes (lane 1) and EBL tumors (lanes 2–13). No quantitative difference between the signals observed for the RNA from normal tissue and the RNAs from EBL tumors was observed. The same conclusion held true for the other *onc* DNA probes tested. Thus it appears that none of the *onc* genes tested were implicated in the maintenance of EBL tumors by a mechanism involving enhanced expression of these genes.

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