Expression of S71-Related Sequences in Human Cells

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Introduction

The presence of C-type retrovirus-related proteins in normal or neoplastic human tissues has been described in several studies (for review see [1]). Antigens related to structural proteins of the simian sarcoma-associated virus (SSAV)/gibbon ape leukemia virus (GaLV) primate retrovirus group have been demonstrated in human leukemic cells [2] and placenta [3]. We have previously reported the isolation of a 70-kDa protein from human leukemic sera that cross-reacts with the capsid proteins (CA) of SSAV and baboon endogenous virus (BaEV) [4]. Furthermore, proteins related to the SSAV envelope gp70 protein (SU) seem to be of value in indicating the prognosis of patients with acute leukemia and chronic myelogenous leukemia (CML) in blast crisis [5]. In search of the origin of these proteins, we have identified about 25–35 SSAV-related sequences in the human genome by low stringency hybridization. One of these sequences, S71, was molecularly cloned and further analyzed [6]. S71 is a truncated retroviral element with the genomic organization 5-gag-SNRS-pol-LTR-3′ [7, 8]. SNRS represents a region of 1130 bp in S71 that consists of nonretroviral sequences. Here, we report our studies on the expression of S71-related sequences in human cell lines and tissues.

Material and Methods

The human leukemia cell line K562 was provided by Dr. Ziegler, University of Munich, FRG. The human placenta complementary deoxyribonucleic acid (cDNA) library in λgt11 was purchased from Clontech, Palo Alto, California, USA. Total cellular ribonucleic acid (RNA) was prepared from tissues and cell lines by the method of Chirgwin et al. [9]. The polyadenylated RNA fraction was purified from total cellular RNA by chromatography on oligo(dT)-cellulose columns. For Northern analysis, polyadenylated RNA was glyoxylated, separated by electrophoresis in agarose gels, and transferred to nylon filters (Zetaprobe, BioRad, Richmond California). Hybridization was carried out at 50 °C in 50% formamide, 1.5 × SSPE (1 × SSPE = 0.18 *M* NaCl, 0.01 *M* Na_2HPO_4, 0.001 *M* EDTA), 1% SDS, 0.5% powdered milk, yeast RNA 0.2 mg/ml, salmon sperm DNA 0.5 mg/ml, and ^{32}P-labeled DNA 1–2 × 10^6 cpm/ml. Filters were washed at 60 °C in 0.1 × SSC (1 × SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate), 1% SDS. Hybridization of Southern blots and phage filter lifts was carried out as described previously [6]. DNA fragments from isolated cDNA clones were subcloned in pUC120. Dideoxy sequencing of double-stranded plasmid DNA was carried out as described [8].

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R. Neth et al. (Eds.)

Results and Discussion

By low-stringency hybridization of human DNA with specific probes of the long terminal repeat (LTR), *gag*, and *pol* regions of S71 we have found S71 to belong to a family of related sequences comprising about 15–20 copies per haploid genome (data not shown). To study expression of these retroviral elements we have screened several human cell lines by Northern blot analysis, using a 3-kb fragment of S71 that contained the 3′ part of SNRS, *pol*, and LTR. The human leukemia cell line K562, derived from a patient with CML in blast crisis, showed a prominent band of 2.9 kb as well as two minor bands of 3.6 and 2.5 kb (Fig. 1, lane 1). These bands could not be detected in normal human peripheral blood cells (lane 2) and other human cell lines (T-cell lymphomas, KE37, H9, HUT78; monocytes, U937; breast carcinoma, T47D; amnion cells, AMA; data not shown).

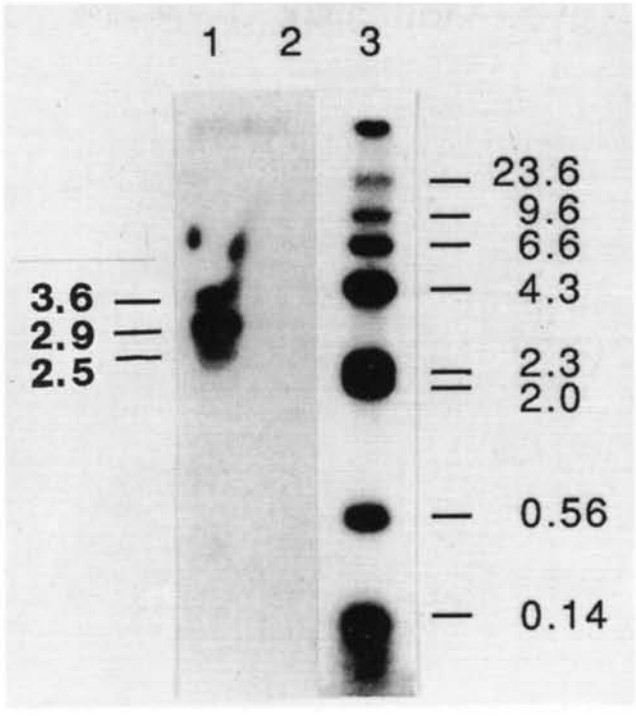

Fig. 1. Expression of S71-related sequences in human leukemic cells. mRNA isolated from human K562 cells (*1*) and normal peripheral blood cells (*2*) were hybridized with a 3-kb S71 SNRS-*pol*-LTR fragment as described in "Material and Methods." *3*, Size marker

High-level expression of human endogenous retroviral elements is often found in placenta, and fetal tissues (for reviews see [1, 10, 11]). Therefore, we screened a human placenta cDNA library under low-stringency hybridization conditions using a recombinant full-length S71 genome as hybridization probe. We isolated a cDNA clone 1.1 kb in length, Pl124, that hybridized specifically only with the S71 LTR, but not with S71 *gag*, *pol*, and SNRS sequences (data not shown). Sequence analysis revealed that a 350-bp stretch of Pl124 shows about 76% homology to the 3′ end of S71 (Fig. 2). The homologous region comprises half of the U3 sequences and is responsible for the strong hybridization signal obtained with S71 LTR. The remaining parts of Pl124 appear to be of nonretroviral origin. A search of the EMBL database revealed that the flanking sequences are not homologous to any known cellular or retroviral sequence. Immediately 3′ of the S71U3-related region, however, sequence elements were detected which resemble transcription signals like CAAT box and TATAA box. Although this region shows no obvious similarity with the U3 region of any known endogenous LTRs, it may have been introduced by a recombination event with another as yet unknown retroviral element. Singular, unrelated subregions within the U3 region of endogenous retroviral LTRs have also been observed in another family of human C-type elements [12]. During cDNA cloning, Pl124 may have lost its original 5-terminus. It is therefore not yet clear whether this sequence is transcribed from a 5′-LTR or a nonretroviral external promoter, as has been shown for other LTR containing retroelements [13].

For further characterization of the nonretroviral sequences of Pl124, we hybridized Southern blots of human genomic DNA with a Pl124 probe under high-stringency conditions (Fig. 3). After digestion with various restriction enzymes, the hybridization patterns of human DNA (lanes 1–5 and 7–11) showed only two or three prominent bands, indicating that the human genome

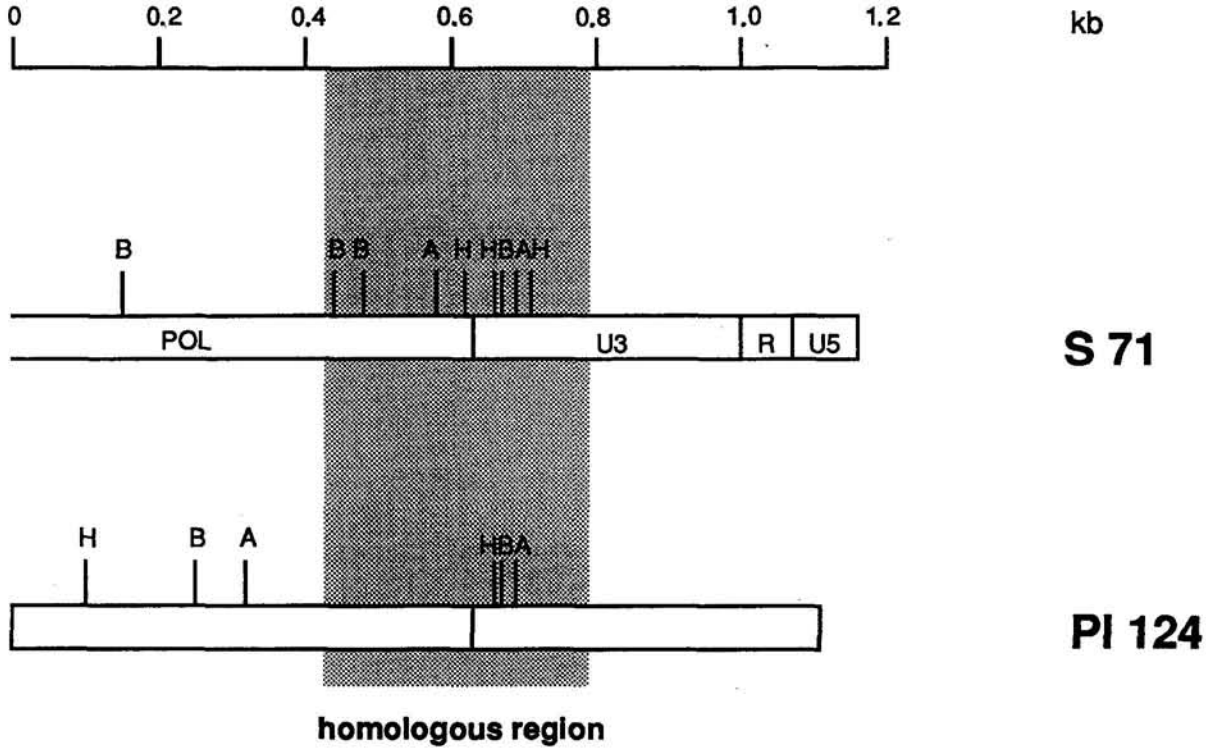

Fig. 2. Restriction map of Pl124 in comparison to the corresponding region of S71. *A, Ava* I; *B, Bam* HI; *H, Hae* I

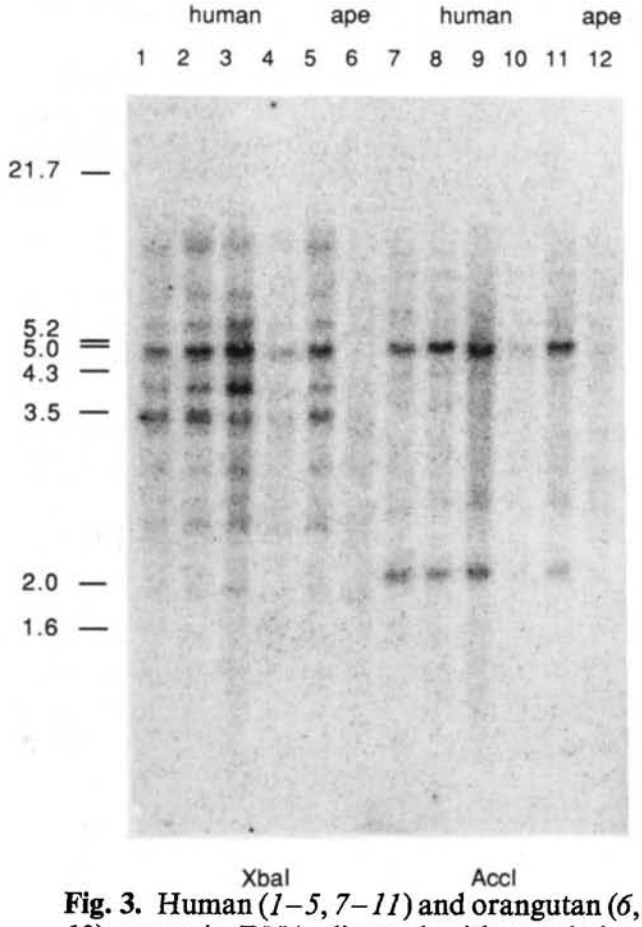

Fig. 3. Human (*1–5, 7–11*) and orangutan (*6, 12*) genomic DNA digested with restriction enzymes *Xba*I or *Acc* I and hybridized to a full-length Pl124 probe under high-stringency conditions

contains only one or a few Pl124 sequences. The appearance of numerous weakly hybridizing fragments is probably due to other S71 LTR-related sequences, including the original genomic S71 LTR, because a similar hybridzation pattern is observed using S71 LTR as hybridization probe (data not shown). Comparative hybridization of orangutan genomic DNA with the Pl124 probe (Fig. 3, lanes 6 and 12) yielded only very weak bands, indicating that orangutan DNA contains only distantly related copies of the Pl124 sequence.

Analysis of expression of several human retroviral elements has shown that transcripts containing retroviral and cellular sequences can be generated by a readthrough mechanism, possibly combined with subsequent splicing [14, 15]. Alternatively, recombination between retroviral and cellular sequences may also occur at the DNA level, as indicated for the SNRS sequence in S71 [8]. Both affect the expression of cellular genes and may therefore be involved in pathogenic events. Further examination of the nature

of retrovirus-related transcripts detected in human neoplastic cells should shed light on the role of endogenous retroviral elements in oncogenesis.

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