provided by Elsevier - Publisher Co

Virology **303,** 164–173 (2002)
doi:10.1006/viro.2002.1615

Characterization of a Novel Human Endogenous Retrovirus, HERV-H/F, Expressed in Human Leukemia Cell Lines

Sebastian Patzke,* Mats Lindeskog,† Else Munthe,* and Hans-Christian Aasheim*,[1]

*The Norwegian Radium Hospital, Department of Immunology, Montebello N-0310 Oslo, Norway; and †Lund University, Department of Medical Microbiology, Section of Virology, Sölvegt. 23, Lund, S-22362 Sweden

Received April 3, 2002; returned to author for revision June 2, 2002; accepted June 19, 2002

We have identified and characterized a human endogenous retrovirus (HERV) *gag* transcript in the human pre-B cell leukemia line Reh. The transcript was found to be a splice product of a structurally intact HERV element located on chromosome 6q13. Its primer binding site is complementary to phenylalanine (F) tRNA, common for the HERV-F family, but the overall genome sequence is closely related to the HERV-H family. The retroviral sequence was therefore designated HERV-H/F. The HERV element shows a distinct mRNA expression pattern among hematopoietic cancer cell lines with expression in some leukemia-derived cell lines of B-lymphoid and myeloid origin. No expression was observed in normal human tissues, indicating a cancer-specific expression pattern. The 5′ long terminal repeat (LTR) was tested for promoter activity in HERV-H/F expressing and nonexpressing cell lines. The cell specificity of the LTR-mediated reporter gene expression did not conclusively correlate with endogenous virus expression, indicating that the transcription regulation of this gene is not alone dependent on cell-specific activity of transcription factors. © 2002 Elsevier Science (USA)

Key Words: HERV; endogenous retrovirus; leukemia; LTR; expression.

INTRODUCTION

Human endogenous retroviruses (HERVs) occupy about 7% of the human genome (Bock and Stoye, 2000) and are very likely footprints of ancient germ cell retroviral infections by exogenous retroviruses that became fixed in the species. Most HERV families entered the genome early in the evolution of primates and were amplified before hominoids separated from Old World monkeys, i.e., 30–45 M years ago (for a review see: Sverdlov, 2000). Today, these sequences constitute an integral part of the human genome and are inherited in a mendelian fashion. However, polymorphism in sequence and chromosomal distribution of some HERVs does exist among the human population (Turner *et al.,* 2001). Phylogenetic and sequence analysis of retroviral elements in the human genome have shown that recombination events between different HERV families may have played a role during the evolution of the human genome (Hughes and Coffin, 2001). At least 22 distinct HERV families have been reported (Tristem, 2000). They are classified by their homology to animal retroviruses and are often subdivided by the degree of *pol* homology or by the primer-binding site (PBS) defining the tRNA species used for priming reverse transcription, i.e., HERV-H utilizing $tRNA^{His}$ or HERV-W utilizing $tRNA^{Trp}$. The different HERV families reside in the genome in varying copy numbers, ranging from single (HERV-R) to 10^3–10^4 (HERV-H) copies per genome (Lower *et al.,* 1996). In addition, there is an even higher number of solitary long terminal repeats (LTRs), products of homologous recombination between the two flanking LTRs of the provirus. Although most of the HERVs are defective due to mutational decay, many of them are transcriptionally active and some HERVs still contain open reading frames (ORFs) for retroviral proteins. There are examples of distinct HERV translation products with potentially important biological functions. HERV-W ENV/syncytin has been implicated in mediation of cell–cell fusion under placental morphogenesis (Blond *et al.,* 2000; Mi *et al.,* 2000) and HERV-K-encoded proteins in complementation of exogenous retroviral proteins like cORF (Magin *et al.,* 1999) and protease (Towler *et al.,* 1998). Expression of potentially immunosuppressive protein-encoding HERV-H *env* transcripts has been detected in various normal and malignant cell types (Lindeskog *et al.,* 1999) and recently, full-length envelope proteins have been shown to have immunosuppressive function *in vivo* (Mangeney *et al.,* 2001). It was shown that expression of the envelope protein of HERV-H in normally rejected tumor cells allows immune escape and proliferation in grafted mice. Other reports have shown that HERV (HERV-K18 *env*)-encoded superantigen (SAg) expression is activated under special conditions such as after EBV infection (Stauffer *et al.,* 2001) or IFN-α stimulation (Sutkowski *et al.,* 2001).

Expression of mRNAs of distinct HERV families has been shown to correlate with several pathologies (ter-

[1] To whom correspondence and reprint requests should be addressed at H-C Aasheim, The Norwegian Radium Hospital, Department of Immunology, Montebello N-0310 Oslo, Norway. Fax: +47 2250 0730. E-mail: hansas@labmed.uio.no.

0042-6822/02 $35.00
© 2002 Elsevier Science (USA)
All rights reserved.

atocarcinomas, tumor cell lines, inflammatory brain diseases, autoimmunity (for review see Urnovitz and Murphy, 1996; Lower, 1999), but also expression in normal tissues has been reported (placenta, peripheral blood mononuclear cells) (for review see Lower *et al.,* 1996). In general, HERV expression is controlled by specific regulatory sequence elements located mainly in the U3 region of the LTR (Schon *et al.,* 2001; Majors, 1990). The LTR provides signals required for transcription initiation and termination, such as transcription factor binding sites, enhancer elements, and polyadenylation signals. Therefore it is possible that some of the solitary LTRs have been adapted as regulatory elements of both sense and antisense transcripts of adjacent genes (Brosius, 1999; Ling *et al.,* 2001).

In this study, we have characterized a novel HERV element, HERV-H/F. This HERV element shows a distinct mRNA expression pattern among hematopoietic cancer cell lines, whereas no expression was found in normal tissues. Here we present and discuss its sequence, phylogeny, expression, and LTR promoter activity.

RESULTS AND DISCUSSION

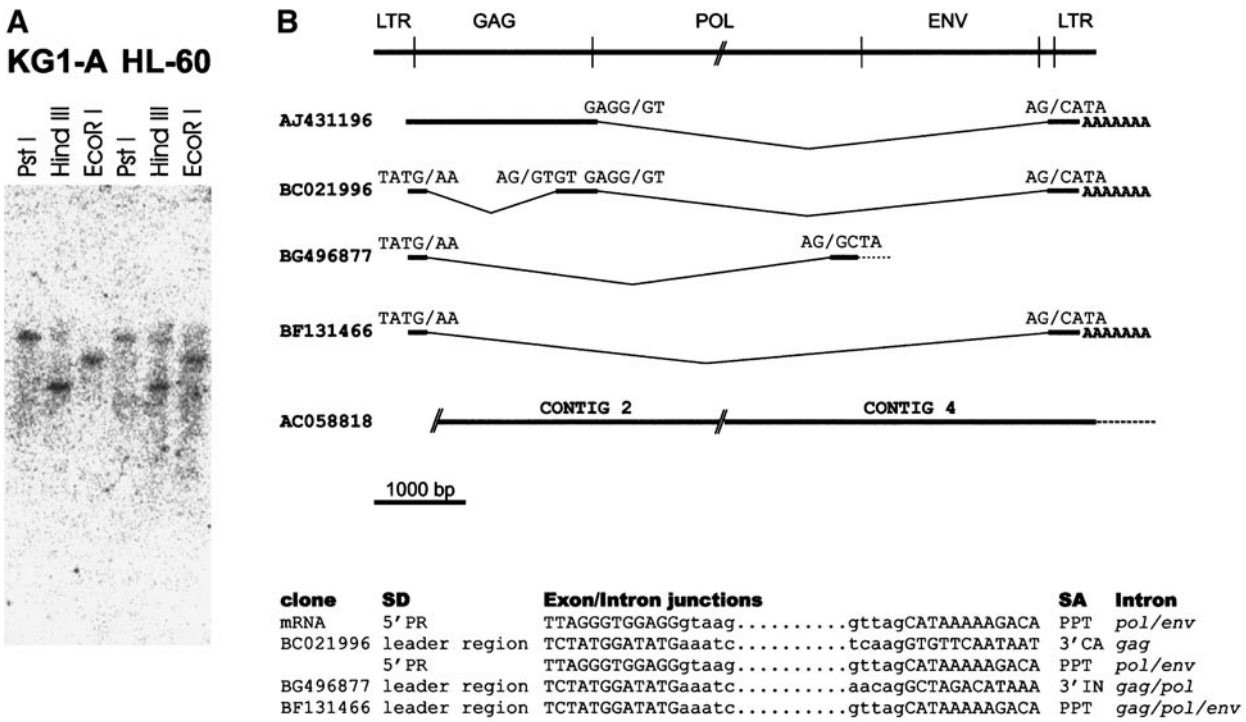
Characterization of a HERV transcript and provirus assembly

A 393 nucleotides (nt)-long cDNA fragment was initially identified by comparing the transcriptome of the human pre-B cell leukemia line Reh with that of the Burkitt lymphoma cell line Bjab by means of cDNA Representational Difference Analysis (Odeberg *et al.,* 2000). For further characterization, a polyadenylated 2238-nt-long cDNA was screened out of a Reh cDNA library using the 393-nt-long cDNA fragment as a probe. Sequence analysis (Accession No. AJ431196) revealed close relationship to the *gag* gene of HERVs of the HERV-H family (approximately 70% homology to human RGH2) (Hirose *et al.,* 1993). The 5′ part of the sequence contains a partial LTR region followed by a PBS with the sequence 5′-TGGTGCCGAAAGCCCGGGAT-3′ almost completely complementary to the final 19 nt of human placenta phenylalanine (F) tRNA (5′-m^{1}AUCCCGGG-UUUCGG-CACCA-3′) (Roe *et al.,* 1975). The *gag* sequence is disrupted by several frameshift mutations. A putative ORF, from nucleotide 227 (ATG) to nucleotide 526 (TAA) in the cDNA sequence encodes a 99 amino acid-long peptide with no homologies to any characterized proteins. The ATG initiation codon is embedded in the context CT-GTCTAUGG with about 50% homology to the Kozak consensus sequences GCCACCAUGG (Kozak, 1984) and was predicted as start codon by the NetStart1.0 software (Pedersen and Nielsen, 1997; http://www.cbs.dtu.dk/services/NetStart). *In vitro* transcription and translation of the cDNA gave no translation product (data not shown), and therefore we cannot state that this open reading frame is translated in cells.

A genomic Southern blot was hybridized under stringent conditions with a probe derived from the *gag* sequence. The results show hybridization to a single band, indicating a single copy of this HERV in the human genome (Fig. 1A). BLAST analysis of annotated human genome sequences revealed a cosmid with Accession No. AC058818 (mapped to chromosome 6q13) to contain the corresponding genomic sequence of our transcript (Fig. 1B). The transcript appeared as a splice product of a not yet described endogenous retroviral element. Supported by the cDNA sequence, we assembled the retroviral element with contigs 2 and 4 of cosmid AC058818, where contig 2 is lacking the 5′ LTR and the very beginning of the *gag* gene, but contains the start of the *pol* gene, and contig 4 includes the rest of the *pol* gene and the complete *env* gene followed by a 3′ LTR with a typical TG . . . CA structure (Temin, 1981). The 3′ LTR is preceded by a putative polypurine tract. We therefore assumed to have identified a structurally intact provirus (5′ LTR-*gag*-*pol*-*env*-3′ LTR; Fig. 1B), even though the available genomic sequence did not include the 5′ LTR. Primers were designed to amplify the 5′ LTR to confirm its existence in genomic DNA. A 489-nt-long fragment was amplified from genomic DNA isolated from the Reh cell line using a 5′ primer identical to the first 18 nt of the 3′ LTR and a 3′ primer complementary to the first 18 nt following the retroviral PBS of the *gag* transcript. The PCR product was cloned and five independent clones were sequenced. All sequences were identical to each other and contained the first 126 nt of the originally isolated *gag* transcript, confirming its association with the provirus, in addition to a LTR sequence (Accession No. AJ431197) homologous to the 3′ LTR (90%, Fig. 2). The complete assembled sequence of the retroviral element is about 8 kb long and displays a structurally intact provirus. A schematic alignment of the genomic structure of the provirus and the isolated related transcripts as well as annotated expressed sequence tag (EST) sequences is depicted in Fig. 1B.

Phylogenetic aspects of the HERV element

As stated above, the primer binding site of this HERV is complementary to phenylalanine-tRNA (tRNA^F), in common with the HERV-F (Kjellman *et al.,* 1999b), HERV-Fb (Tristem, 2000), and HERV-XA34 (Kjellman *et al.,* 1999a) families and elements enclosed by LTR46 (unpublished observation). But strikingly, the identified HERV sequence is more related to the HERV-H family showing approximately 70% nt identity in the *gag* gene region, 75% nt identity in *pol* and *env* gene regions, and 86% in the R region of the LTR. Phylogenetic analysis of the *pol* gene shows low relationship of the novel HERV to the other HERV-F family members, but places it into a cluster belonging to the HERV-H family. Interestingly, this cluster is made up by a subfamily of ancient HERV-H family



clone	SD	Exon/Intron junctions	SA	Intron
mRNA	5′PR	TTAGGGTGGAGGgtaag.....gttagCATAAAAAGACA	PPT	*pol/env*
BC021996	leader region	TCTATGGATATGaaatc.....tcaagGTGTTCAATAAT	3′CA	*gag*
	5′PR	TTAGGGTGGAGGgtaag.....gttagCATAAAAAGACA	PPT	*pol/env*
BG496877	leader region	TCTATGGATATGaaatc.....aacagGCTAGACATAAA	3′IN	*gag/pol*
BF131466	leader region	TCTATGGATATGaaatc.....gttagCATAAAAAGACA	PPT	*gag/pol/env*

FIG. 1. Genomic Southern blot, sequence assembly and splice pattern. (A) Southern blot analysis with a 393-nt *gag* probe hybridized to fragmented genomic DNA from human pre-B leukemia cell line Reh (HERV-H/F-expressing) and promyeloid leukemia cell line HL-60 (-nonexpressing). (B) Schematic presentation of the assembled HERV-H/F sequence and alignment of the isolated *gag* mRNA and reported GenBank entries. Splice donor (SD) and acceptor (SA) sites are indicated. Slashes indicate gaps of unknown length.

members located within Accessions Nos. AC004510, AC002384, U95626, and AL354751 with 94.4, 93.9, 91.6, and 91.9% identity, respectively, to 5′ and 3′ LTR (unpublished elements found in indicated GenBank entries). All these elements share similar integration dates and phenylalanine tRNA PBSs (Fig. 3). We therefore suggest that the novel HERV element be designated HERV-H/F. The HERV-H/F cluster also includes sequences (AC002384, AL354751, and HERV-H/F) with full-length *gag* genes, in contrast to the HERV-H elements like RGH2, which bear large deletions in this region. The phylogenetic analysis may indicate a PBS shift inside the HERV-H "superfamily," a phenomenon reported for the murine retrovirus like VL30 family (Itin and Keshet, 1985).

The *env* gene encompasses a region (Accession No. AC058818, contig 4 nt 11660–11610) coding for a sequence (LQNRQGLDLLTADKGGL) highly homologous to the immunosuppressive peptide found in HERV-H19 (LQNRRGCDLLTAEKGGL) and other retroviruses (Lindeskog *et al.,* 1999). Although no ORF exists in frame with the immunosuppressive *env* region, one cannot exclude the possibility that a homologous peptide may be synthesized by mutational or translational −1 frameshifting in an A-rich region in the *env* gene, thus promoting the synthesis of 25-kDa protein. However, this possibility remains speculative as long as no further experimental evidence is presented.

The 5′ LTR (446 bp; Accession No. AJ431197) and the 3′ LTR (449 bp; Accession No. AC058818, contig 4 nt 11107–10659) of HERV-H/F share about 90% nucleotide identity (402 identities shared on 446 nt; Fig. 2). Assuming an average mutation rate of 0.13% per million years (Sverdlov, 2000), the integration date of the virus may be estimated to 35–40 M years ago, that is, before the hominoid lineage diverged from the Old World apes (approx. 30 M years). As presented in Fig. 1A, the complete HERV element is present in a single copy. Homology searches with the 5′ LTR sequence against the Human Genome Sequence Database revealed at least 12 closely related LTRs distributed on chromosomes 1, 4, 6, 8, 9, 13, and X (Table 1). The relationship is outlined by the alignment in Fig. 4. Three of the homologous solitary LTRs are located in antisense direction in introns of annotated genes (Table 1). Whether their presence affects regulation of these genes remains unknown.

In conclusion, due to the closer relationship to the HERV-H family of endogenous retroviruses and the presence of a $tRNA^{Phe}$ primer binding site, we suggest that the novel HERV element identified on chromosome 6 be designated HERV-H/F.

HERV-H/F mRNA expression analysis

Following the initial observation of expression in the pre-B leukemia cell line Reh, Northern blot analysis on selected cancer cell lines of hematopoietic origin was performed. Hybridization with the isolated cDNA, consti-

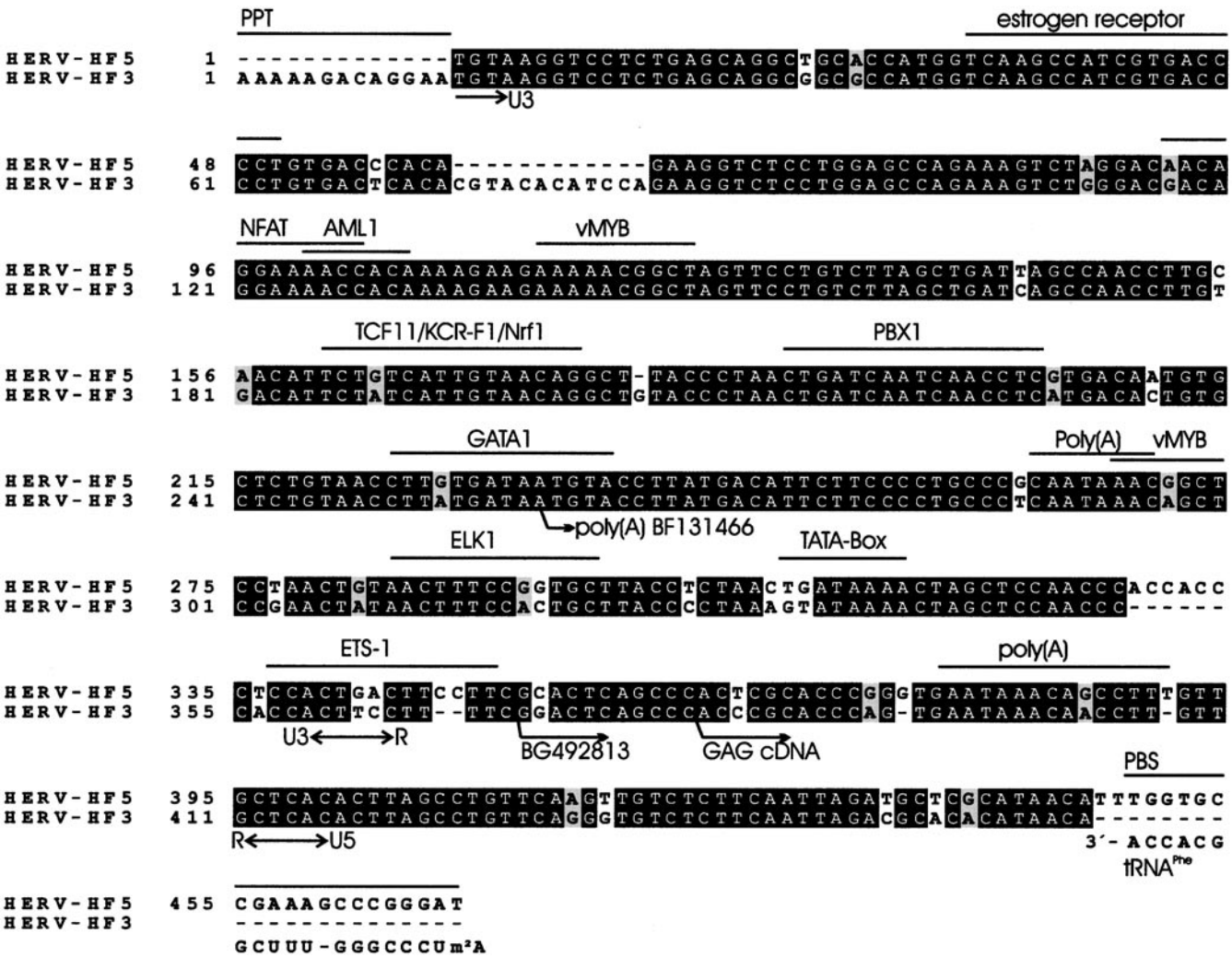


FIG. 2. LTR analysis. Alignment of 5′ and 3′ LTR of the HERV-H/F element on chromosome 6. The LTRs share about 91% sequence identity. Polypurine tract (PPT) and primer binding site (PBS) are indicated. Potential transcription factor binding sites as detected by MatInspector Release Professional 5.2 (Quandt *et al.,* 1995) are highlighted.

tuting a *gag* specific probe, showed expression in only five cell lines: in the two B-lymphoid cell lines Reh and Nalm-6, both derived from acute lymphoblastic leukemia (ALL), and in the three promyeloid cell lines KG1A, K562, and U937 (Fig. 5). No expression could be detected in two other B-cell leukemia lines (Tom-1 and BV173), in three B-lymphoma cell lines (Daudi, Bjab, and U266), in the myeloid leukemia cell line HL-60, or in any of the three T-lymphoid leukemia cell lines (HPB-ALL, JM, Jurkat). Northern blot analysis of poly(A^+) mRNA reflecting 24 normal human tissues showed no hybridization signal (data not shown). Three hybridizing transcripts are observed with the *gag* probe, most probably reflecting the full-length transcript (about 8 kb) and two splice products located between 18S and 28S rRNA (Fig. 5). Hybridization with a PCR-amplified 1557 nt probe specific for the *env* gene (see Material and Methods) showed an expression pattern similar to that shown for the *gag* probe (Fig. 5), further supporting the sequence assembly of contigs 2 and 4 from cosmid AC058818. The alignment of identical EST sequences with the HERV-H/F provirus also identified several splice variants which may be divided into two classes by their 5′ splice donor site (Fig. 1B). The identified polyadenylated *gag* transcript with spliced out *pol* and *env* genes as a GT-AG intron defines the first class, whereas the second class uses a noncanonical splice site to remove AA-AG introns from the pre-mRNA/proviral transcript. The noncanonical splice donor is located in the leader region between 5′ LTR and *gag,* as typically seen in mammalian type C exogenous retroviruses. Three alternative splice acceptor sites could be identified in reported GenBank EST entries, located in the 3′ region of the *gag* gene (Accession No. BC021996), at the 3′ region of the *pol* gene (Accession No. BG496877), and close to the polypurine tract in front of the 3′ LTR (Accession No. BF131466).

Interestingly, the 3′ splice acceptor site in the *pol* gene locates 129 nucleotides upstream relative to the TGG

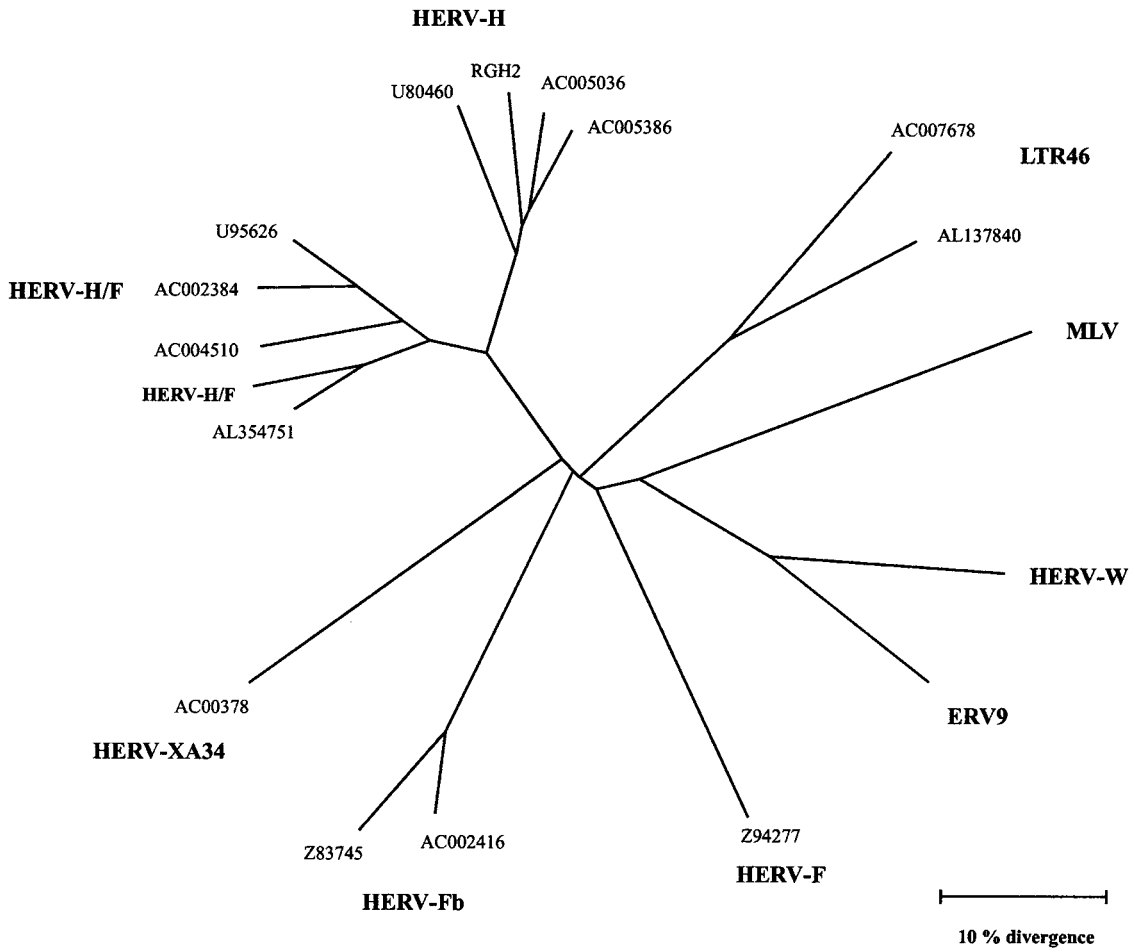


FIG. 3. Phylogenetic analysis. Unrooted tree of an alignment of a conserved sequence region of the HERV-H/F *pol* gene (Accession No. AC058818, contig 2 nt 900–274).

codon of the first base of the conserved WTGS motif in the C-terminal part of the integrase, which is similar to the splice acceptor sites reported for other retroviruses. The complex splice pattern found for the HERV-H/F element appears similar to the alternative splice pattern of the HERV-H family found in T-cell leukemia and normal

TABLE 1

Identified HERV-H/F LTRs in the Available Human Genome Database

BAC clone	Length (nt)	Locus	% Identity/% gaps	Remarks
AC025699	461	1q43-44	83/5	
AC024669	474	4p15	83/7	Located in intron of CALP (Unigene: Hs.193323)
AC080031	466	4q21.1-3	85/8	
Z84476	471	6p21-22	85/7	
HERV-H/F 5′ LTR	446	6q13		
AC058818 (HERV-H/F 3′ LTR)	449	6q13	88/5	
AC022058	471	8	84/7	
AP002906	454	8q22-23	84/6	Located in intron of LC27 (Unigene: Hs.296398)
AL353753a	458	9p13	84/7	5′ and 3′ LTR of 5409-nt-long HERV-H/F homolog
AL353753b	459	9p13	84/6	
AL354751	468	9p22	84/6	Located in intron of SPTLC1 (Unigene: Hs.90458)
AL354852	471	13	84/7	
AL390875	454	Xq25-26	85/6	

Note. LTRs are listed by their representing BAC clone. Homology of the respective LTRs to the HERV-H/F 5′LTR reported in this study is indicated by the percentage of identity and gaps as determined by Clustal W1.81.

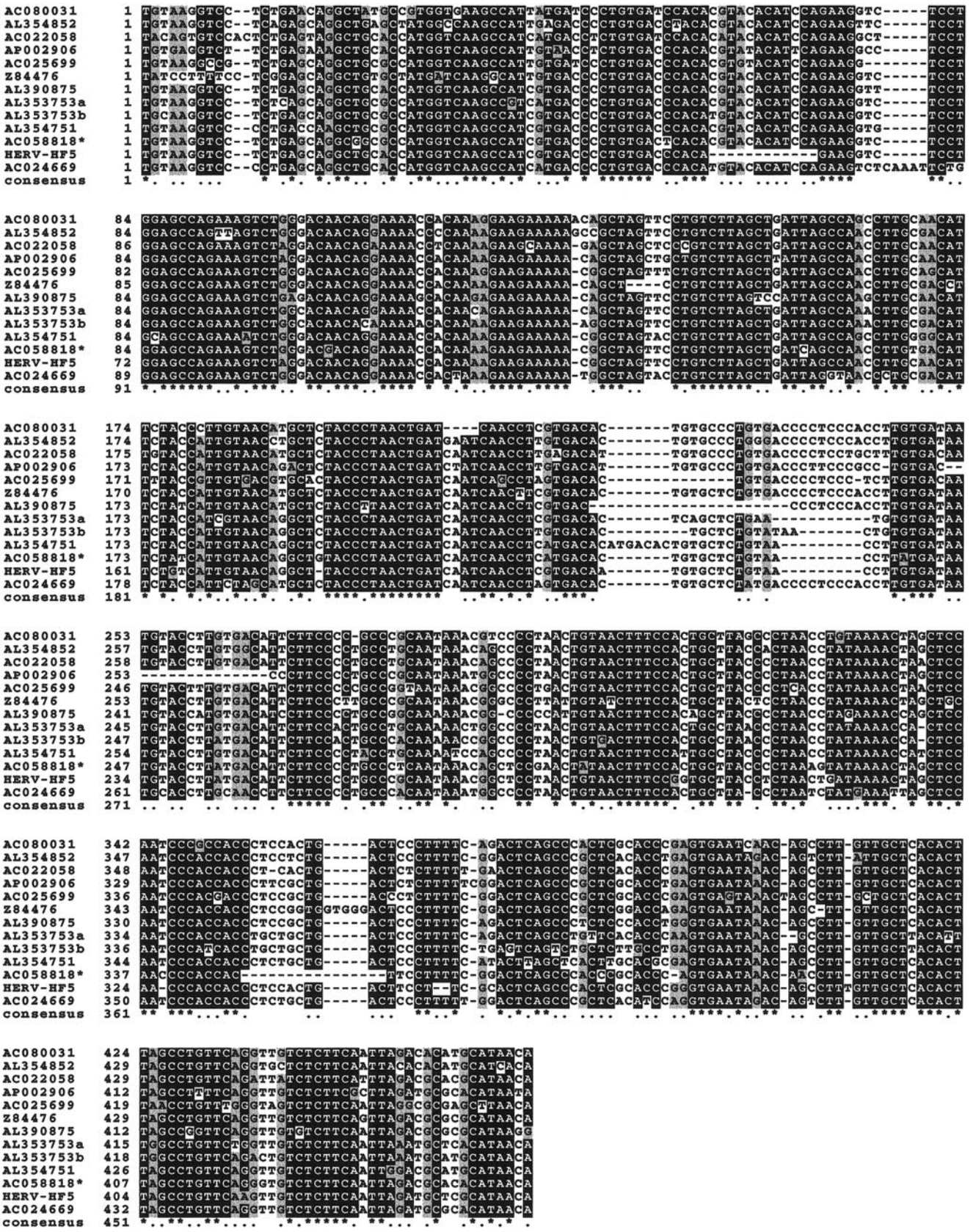


FIG. 4. Alignment of LTR homologues identified by homology searches against human genome database. LTRs are entitled by their presenting cosmid (see Table 2); 5′ and 3′ LTRs of the full-length HERV-H/F element are marked HERV-H/F5 and AC058818*, respectively.

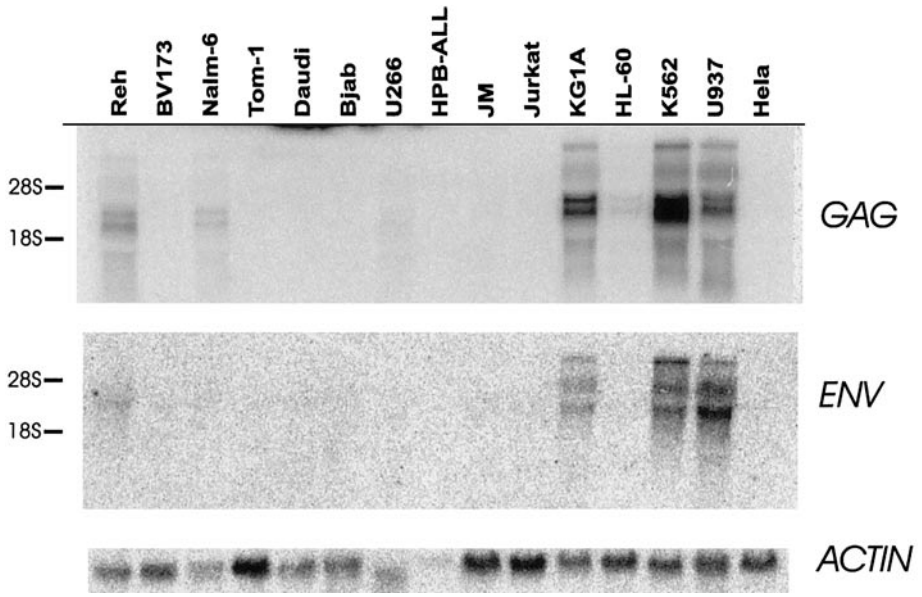


FIG. 5. HERV-H/F mRNA expression. Hybridization with probes specific for *gag* and *env* of HERV-H/F against total RNA from selected human hematopoietic cancer cell lines (B-lineage: Reh, BV173, Nalm-6, Tom-1, Daudi, Bjab, U266; T-lineage: HPB-ALL, JM, Jurkat; myeloid lineage: KG1A, HL-60, K562, U937, and the cervix carcinoma cell line HeLa).

leukocytes (Lindeskog and Blomberg, 1997), supporting the idea of a regulatory function of alternative splicing in these ancient infectious progenitors as found in HERV (Lower *et al.,* 1993) and in other retrovirus genera like lentiviruses, HTLV-like viruses, and spumaviruses (Rabson and Graves, 1997). These findings support the mRNA expression data showing hybridization to several transcripts with both *gag* and *env* probes. None of the concomitant human EST database GenBank entries are derived from normal human tissues, but all were isolated from cancer cell lines derived from various tissues (Table 2). The distinct expression pattern among hematopoietic cancer cell lines and reported GenBank entries suggests a cancer-specific expression of our reported HERV-H/F transcripts.

All expression pattern data presented here are based on Northern blot analyses and all HERV-H/F-expressing leukemia cell lines are reflecting early stages of B and myeloid lineage differentiation taking place in the bone marrow. We cannot rule out the possibility that HERV-H/F is expressed in normal cells of these differentiation stages, since these are present only in low numbers in normal human bone marrow or peripheral blood leukocytes. Nonetheless, both the HERV-H/F-expressing cell line Reh and the nonexpressing cell line HL-60 show a single band of identical size in the genomic Southern blot (Fig. 1A), indicating that the cell specificity of HERV-H/F gene expression is a result of gene regulation rather than gene amplification.

5′ LTR promoter activity

To further characterize the regulation of HERV-H/F transcription, its 5′ LTR was analyzed for potential tran-

TABLE 2

Selected HERV-H/F GenBank Human EST Database Entries

Human tissue library Mammalian gene collection (MGC)	Tissue	Cancer cell line origin	Genbank Accession No.
NIH_MGC60	Prostate	Adenocarcinoma	BG500808, BG498497
NIH_MGC91	Prostate	Adenocarcinoma	BG284793, BG283193
NIH_MGC59	Lung	Mucoepidermoid carcinoma	BG496877, BG776076
NIH_MGC55	Bone marrow	Acute myeloid leukemia	BF243442, BE748890
NIH_MGC54	Bone marrow	Chronic myeloid leukemia	BC021996, BF211467
NIH_MGC72	Skin	Melanotic melanoma	BE891699
NIH_MGC58	Kidney	Hypernephroma	BF131466
NIH_MGC61	Testis	Embryonal carcinoma	BG529567

Note. Human ESTs identical to the reported HERV-H/F sequence on 6q13 are all derived from cell line cDNA libraries of the Mammalian gene collection (http://mgc.nci.nih.gov/). Origin of representative GenBank EST entries are indicated.

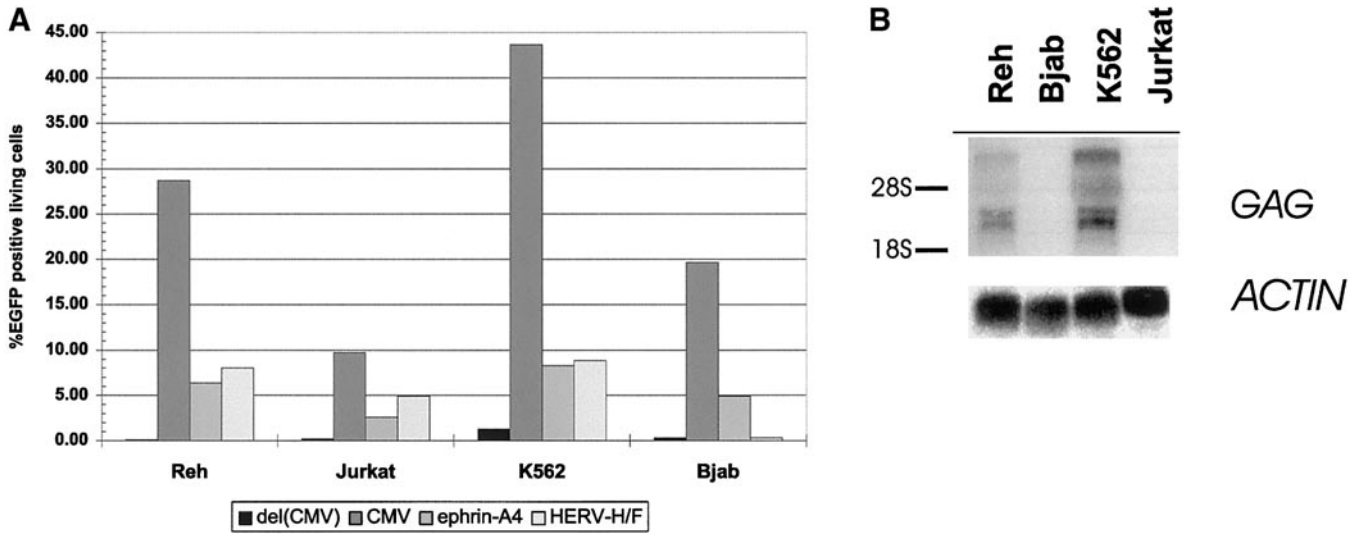


FIG. 6. LTR promoter activity. (A) LTR-mediated EGFP reporter gene expression in HERV-H/F-expressing (Reh, K562) and -nonexpressing cell lines (Bjab, Jurkat). For comparison, EGFP expression of a promoterless (del(CMV)), CMV promoter, and ephrin-A4 promoter construct were tested in parallel. Results shown are from a representative experiment of three. (B) The same cell lines were tested for endogenous HERV-H/F mRNA expression by Northern blot analysis.

scription factor binding sites and tested for promoter activity in a reporter gene assay. Many of the predicted potential transcription factor binding sites found in the 5′ LTR of HERV-H/F are related to transcription factors (GATA-1, vMYB, AML1, PBX1, ETS-1, ELK-1; Fig. 2) involved in normal hematopoiesis and with aberrant expression in B and myeloid lineage leukemia (Crans and Sakamoto, 2001).

The LTR with its adjacent primer binding site was cloned in front of the gene encoding green fluorescent protein (EGFP) and transiently transfected into the human cancer cell lines Reh, Bjab, Jurkat, and K562. The reporter gene activity was evaluated by flow cytometric analysis of EGFP expression in living cells (Fig. 6). Although this reporter gene assay does not allow a quantitative analysis of promoter activity, its sensitivity is adequate to receive a qualitative result. In order to evaluate the promoter activity of the LTR, a promoterless construct (background) and constructs bearing EGFP under the control of the CMV or the ephrin-A4 promoter were tested in parallel. The expression of the receptor tyrosine kinase ligand ephrin-A4 in the examined cell lines has been reported earlier by our laboratory (Aasheim *et al.,* 2000) and its promoter has been characterized recently (Munthe and Aasheim, submitted). Although *gag* transcripts were detectable only in Reh and K562 cells, EGFP expression, under the control of the 5′ LTR, was observed in Reh, K562, and Jurkat cells (Fig. 6). No EGFP expression was detected in the *gag*-negative cell line Bjab. The results indicate that the cell-specific expression of HERV-H/F may involve additional control mechanisms and is not regulated only by cell-specific activity of transcription factors. This may be achieved by repressor elements binding outside the 5′ LTR and/or by general genomic silencing mechanism like modulation of chromatin structure, methylation, or acetylation.

Because of the cell type-specific expression of many reported HERVs, gene therapy research has drawn attention to the LTRs as versatile tools for the construction of targeted retroviral expression vectors (Schon *et al.,* 2001). The LTR-mediated EGFP expression in Jurkat cells emphasizes that the transfer of HERV LTRs into gene therapeutic applications deserves special consideration.

Conclusions

The detection of a *gag*-related transcript in the human leukemia cell line Reh led to the identification of a member of a new HERV-H subfamily, designated HERV-H/F and located on chromosome 6q13. Expression of unspliced and spliced subgenomic HERV-H/F mRNAs could be detected only in B and myeloid lineage leukemia cell lines trapped at early stages of differentiation, but expression in other malignant cell lines has also been reported. The expression may be cancer related since no expression so far has been detected in normal human tissues. The 5′ LTR driving the transcription was tested for promoter activity and the expression pattern of HERV-H/F did not conclusively correspond to the promoter activity in transfected cells. A biological significance of the observed expression of HERV-H/F remains unclear and lies beyond the scope of our presented characterization.

MATERIAL AND METHODS

Cell culture

The following human cell lines were used in this study: pro-B-cell lines Tom-1 (Okabe *et al.,* 1987) and BV173

(DSZM ACC20), pre-B-cell lines Reh (ATCC CRL 8286) and Nalm 6; mature B-cell lines Bjab (Dr. G. Moldenhauer, University of Heidelberg, Heidelberg, Germany) and Daudi (ATCC CCL 213); plasmacytoid cell line U266 (ATCC TIB 196); T-cell lines JM (ECACC 86010201), Jurkat (ATCC TIB 152), and HPB ALL (DSZM ACC 483); myeloid cell lines KG1-A (ATCC CCL 246); HL-60 (ATCC CCL 240) and U937 (ATCC CRL-1596); erythroid precursor cell line K562 (ATCC CCL-243); and cervical carcinoma cell line HeLa (ATCC CCL 2). All cell lines were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics at 37°C in a humidified atmosphere with 5% CO_2.

RNA isolation and Northern blot analysis

Total RNA from cell lines was isolated by standard methods (Chomczynski and Sacchi, 1987). For Northern blot analysis 15 μg total RNA was separated (1% agarose gel) under denaturing conditions and transferred to Hybond-N+ membrane (Amersham Biosciences Ltd., UK). All hybridizations were performed at 65°C. Probes were [α^{32}P]dCTP labeled using the rediprime II random prime labeling system (Amersham Biosciences). Blots were washed stringently, and hybridization was evaluated by autoradiography using the Storm 860 PhosphorImaging system (Amersham Biosciences). Commercially available Northern tissue blots were obtained from Clontech (MTN I and I; Clontech, CA).

Genomic DNA isolation and Southern blot hybridization

Genomic DNA was isolated from cell lines using the QIAamp DNA blood mini kit (QIAGEN GmbH, Germany). For Southern blotting 10 μg of genomic DNA was digested by incubation overnight at 37°C with the restriction endonucleases *Pst*I, *Hin*dIII, or *Eco*RI, respectively, using the supplied buffer solutions in presence of 4 mM spermidine. DNA digests were separated overnight on an 0.8% agarose gel and blotted to Hybond N+ membrane (Amersham Biosciences). Probe labeling and hybridization were performed as described for Northern blot analysis.

First-strand cDNA synthesis and PCR

Poly(A^+) mRNA was isolated from total RNA using oligo(dT) beads, and first-strand cDNA was synthesized directly on mRNA bound to oligo(dT), as described previously (Aasheim *et al.,* 1994). Finally the first-strand cDNA beads were washed twice in 100 μl TE buffer, solved in 25 μl TE, and stored at −20°C. All PCR reactions were performed under the following final conditions: 10 mM Tris–Cl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl (adjusted with 10× buffer); 0.16 mM dNTP (Amersham Biosciences); 0.02 U/μl Taq polymerase; 0.1 μM each primer). The 393-nt *gag* probe was PCR amplified from the isolated *gag* cDNA (Accession No. AJ431196) clone using the primers 5′-TTGTGGGACTTAGACAACTTC-3′ (forward) and 5′-TTTGGTTCCTGCCACCTCTC-3′ (reverse). The 1547-nt *env* probe was PCR amplified from 1 μl cDNA from KG1A cells in a 50-μl reaction using the forward primer 5′-CAATCCTTCCTATGCATTTCC-3′ and the reverse primer 5′-CTAAGCTAGAGAATGGCCAGG-3′, resulting in a single PCR product. The 5′ LTR was PCR amplified from 2 μg genomic DNA from Reh cells using forward primer 5′-TGTAAGGTCCTCTGAGCA-3′ and reverse primer 5′-GTCTGCCGGAGGAGTTCC-3′. The PCR product was subcloned into pGEM-T Easy Vector System (Promega Corp.) *Ase*I and *Xho*I endonuclease restriction sites at the ends of the LTR for directional cloning into the pEGFP-N3 vector were introduced by PCR on this clones using primers 5′-CCCATTAATTGTAAGGTCCTCTGAGCA-3′ and 5′-CCCCTCGAGGTCTGCCGGAGGAGTTCC-3′.

Green fluorescence protein (EGFP) reporter gene assay

For analysis of promoter activity, the CMV promoter of the EGFP gene in the pEGFP-N3 vector (Clontech Laboratories) was removed using the restriction endonucleases *Ase*I and *Xho*I (New England Biolabs, Inc.) and replaced by either the HERV-H/F 5′ LTR or the ephrinA4 promoter. As background control, promoterless pEGFP-N3 vector was constructed by filling in overhang with Klenow polymerase and religation of the blunt-ended vector. Transient transfection of cells was performed by electroporation. Exponentially growing cells (0.5–1 10^6/ml) were harvested and resuspended in RPMI 1640 and kept on ice; 5 μg of the different promoter constructs was added to 400-μl cells (1–2 10^6/ml). Electroporation was conducted at 200 V for 70 ms (Reh, Jurkat), 30 ms (K562), and 10 ms (BJAB), respectively, using an ECM 830 Electro Square Porator (BTX, Inc., San Diego, CA). After electroporation, 800 μl of conditioned medium supplemented with 5% FBS was added immediately, and the cells were incubated at 37°C overnight. EGFP expression was monitored by FACS analysis of 2×10^4 living cells.

ACKNOWLEDGMENT

This work was supported by The Norwegian Cancer Society.

REFERENCES

Aasheim, H. C., Deggerdal, A., Smeland, E. B., and Hornes, E. (1994). A simple subtraction method for the isolation of cell-specific genes using magnetic monodisperse polymer particles. *Biotechniques* **16,** 716–721.

Aasheim, H. C., Munthe, E., Funderud, S., Smeland, E. B., Beiske, K., and Logtenberg, T. (2000). A splice variant of human ephrin-A4 encodes a soluble molecule that is secreted by activated human B lymphocytes. *Blood* **95,** 221–230.

Blond, J. L., Lavillette, D., Cheynet, V., Bouton, O., Oriol, G., Chapel-Fernandes, S., Mandrand, B., Mallet, F., and Cosset, F. L. (2000). An envelope glycoprotein of the human endogenous retrovirus HERV-W

is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. *J. Virol.* **74,** 3321–3329.

Bock, M., and Stoye, J. P. (2000). Endogenous retroviruses and the human germline. *Curr. Opin. Genet. Dev.* **10,** 651–655.

Brosius, J. (1999). Genomes were forged by massive bombardments with retroelements and retrosequences. *Genetica* **107,** 209–238.

Chomczynski, P., and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162,** 156–159.

Crans, H. N., and Sakamoto, K. M. (2001). Transcription factors and translocations in lymphoid and myeloid leukemia. *Leukemia* **15,** 313–331.

Hirose, Y., Takamatsu, M., and Harada, F. (1993). Presence of env genes in members of the RTVL-H family of human endogenous retrovirus-like elements. *Virology* **192,** 52–61.

Hughes, J. F., and Coffin, J. M. (2001). Evidence for genomic rearrangements mediated by human endogenous retroviruses during primate evolution. *Nat. Genet.* **29,** 487–489.

Itin, A., and Keshet, E. (1985). Primer binding sites corresponding to several tRNA species are present in DNAs of different members of the same retrovirus-like gene family (VL30). *J. Virol.* **54,** 236–239.

Kjellman, C., Sjogren, H. O., Salford, L. G., and Widegren, B. (1999a). HERV-F (XA34) is a full-length human endogenous retrovirus expressed in placental and fetal tissues. *Gene* **239,** 99–107.

Kjellman, C., Sjogren, H. O., and Widegren, B. (1999b). HERV-F, a new group of human endogenous retrovirus sequences. *J. Gen. Virol.* **80,** 2383–2392.

Kozak, M. (1984). Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. *Nucleic Acids Res.* **12,** 857–872.

Lindeskog, M., and Blomberg, J. (1997). Spliced human endogenous retroviral HERV-H env transcripts in T-cell leukaemia cell lines and normal leukocytes: Alternative splicing pattern of HERV-H transcripts. *J. Gen. Virol.* **78,** 2575–2585.

Lindeskog, M., Mager, D. L., and Blomberg, J. (1999). Isolation of a human endogenous retroviral HERV-H element with an open env reading frame. *Virology* **258,** 441–450, doi:10.1006/viro.1999.9750.

Ling, V., Wu, P. W., Finnerty, H. F., Agostino, M. J., Graham, J. R., Chen, S., Jussiff, J. M., Fisk, G. J., Miller, C. P., and Collins, M. (2001). Assembly and annotation of human chromosome 2q33 sequence containing the CD28, CTLA4, and ICOS gene cluster: Analysis by computational, comparative, and microarray approaches. *Genomics* **78,** 155–168.

Lower, R. (1999). The pathogenic potential of endogenous retroviruses: Facts and fantasies. *Trends Microbiol.* **7,** 350–356.

Lower, R., Boller, K., Hasenmaier, B., Korbmacher, C., Muller-Lantzsch, N., Lower, J., and Kurth, R. (1993). Identification of human endogenous retroviruses with complex mRNA expression and particle formation. *Proc. Natl. Acad. Sci. USA* **90,** 4480–4484.

Lower, R., Lower, J., and Kurth, R. (1996). The viruses in all of us: Characteristics and biological significance of human endogenous retrovirus sequences. *Proc. Natl. Acad. Sci. USA* **93,** 5177–5184.

Magin, C., Lower, R., and Lower, J. (1999). cORF and RcRE, the Rev/Rex and RRE/RxRE homologues of the human endogenous retrovirus family HTDV/HERV-K. *J. Virol.* **73,** 9496–9507.

Majors, J. (1990). The structure and function of retroviral long terminal repeats. *Curr. Top. Microbiol. Immunol.* **157,** 49–92.

Mangeney, M., de Parseval, N., Thomas, G., and Heidmann, T. (2001). The full-length envelope of an HERV-H human endogenous retrovirus has immunosuppressive properties. *J. Gen. Virol.* **82,** 2515–2518.

Mi, S., Lee, X., Li, X., Veldman, G. M., Finnerty, H., Racie, L., LaVallie, E., Tang, X. Y., Edouard, P., Howes, S., Keith, J. C., Jr., and McCoy, J. M. (2000). Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* **403,** 785–789.

Odeberg, J., Wood, T., Blucher, A., Rafter, J., Norstedt, G., and Lundeberg, J. (2000). A cDNA RDA protocol using solid-phase technology suited for analysis in small tissue samples. *Biomol. Eng.* **17,** 1–9.

Okabe, M., Matsushima, S., Morioka, M., Kobayashi, M., Abe, S., Sakurada, K., Kakinuma, M., and Miyazaki, T. (1987). Establishment and characterization of a cell line, TOM-1, derived from a patient with Philadelphia chromosome-positive acute lymphocytic leukemia. *Blood* **69,** 990–998.

Pedersen, A. G., and Nielsen, H. (1997). Neural network prediction of translation initiation sites in eukaryotes: Perspectives for EST and genome analysis. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **5,** 226–233.

Rabson, A. B., and Graves, B. J. (1997). Synthesis and processing of viral RNA. *In* "Retroviruses" (J. M. Coffin, S. H. Hughes, and H. E. Varmus, Eds.), pp. 205–262. CSH Laboratory Press, New York.

Roe, B. A., Anandaraj, M. P., Chia, L. S., Randerath, E., Gupta, R. C., and Randerath, K. (1975). Sequence studies on tRNAPhe from placenta: Comparison with known sequences of tRNAPhe from other normal mammalian tissues. *Biochem. Biophys. Res. Commun.* **66,** 1097–1105.

Schon, U., Seifarth, W., Baust, C., Hohenadl, C., Erfle, V., and Leib-Mosch, C. (2001). Cell type-specific expression and promoter activity of human endogenous retroviral long terminal repeats. *Virology* **279,** 280–291, doi:10.1006/viro.2000.0712.

Stauffer, Y., Marguerat, S., Meylan, F., Ucla, C., Sutkowski, N., Huber, B., Pelet, T., and Conrad, B. (2001). Interferon-alpha-induced endogenous superantigen: A model linking environment and autoimmunity. *Immunity* **15,** 591–601.

Sutkowski, N., Conrad, B., Thorley-Lawson, D. A., and Huber, B. T. (2001). Epstein–Barr virus transactivates the human endogenous retrovirus HERV-K18 that encodes a superantigen. *Immunity* **15,** 579–589.

Sverdlov, E. D. (2000). Retroviruses and primate evolution. *Bioessays* **22,** 161–171.

Temin, H. M. (1981). Structure, variation and synthesis of retrovirus long terminal repeat. *Cell* **27,** 1–3.

Towler, E. M., Gulnik, S. V., Bhat, T. N., Xie, D., Gustschina, E., Sumpter, T. R., Robertson, N., Jones, C., Sauter, M., Mueller-Lantzsch, N., Debouck, C., and Erickson, J. W. (1998). Functional characterization of the protease of human endogenous retrovirus, K10: Can it complement HIV-1 protease? *Biochemistry* **37,** 17137–17144.

Tristem, M. (2000). Identification and characterization of novel human endogenous retrovirus families by phylogenetic screening of the human genome mapping project database. *J. Virol.* **74,** 3715–3730.

Turner, G., Barbulescu, M., Su, M., Jensen-Seaman, M. I., Kidd, K. K., and Lenz, J. (2001). Insertional polymorphisms of full-length endogenous retroviruses in humans. *Curr. Biol.* **11,** 1531–1535.

Urnovitz, H. B., and Murphy, W. H. (1996). Human endogenous retroviruses: Nature, occurrence, and clinical implications in human disease. *Clin. Microbiol. Rev.* **9,** 72–99.