Journal of Virology

Comprehensive Analysis of Human Endogenous Retrovirus Transcriptional Activity in Human Tissues with a Retrovirus-Specific Microarray

Wolfgang Seifarth, Oliver Frank, Udo Zeilfelder, Birgit Spiess, Alex D. Greenwood, Rüdiger Hehlmann and Christine Leib-Mösch

J. Virol. **2005, 79(1):341. DOI: 10.1128/JVI.79.1.341-352.2005.**

JOURNAL OF VIROLOGY, Jan. 2005, p. 341–352 Vol. 79, No. 1
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.1.341–352.2005

Comprehensive Analysis of Human Endogenous Retrovirus Transcriptional Activity in Human Tissues with a Retrovirus-Specific Microarray

Wolfgang Seifarth,[1]*† Oliver Frank,[1]† Udo Zeilfelder,[1] Birgit Spiess,[1] Alex D. Greenwood,[2,3] Rüdiger Hehlmann,[1] and Christine Leib-Mösch[1,2]

Medical Clinic III, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim,[1] *GSF-National Research Center for Environment and Health, Institute of Molecular Virology, Neuherberg,*[2] *and Technical University of Munich, Institute of Virology, Munich,*[3] *Germany*

Received 5 May 2004/Accepted 19 August 2004

Retrovirus-like sequences account for 8 to 9% of the human genome. Among these sequences, about 8,000 *pol*-containing proviral elements have been identified to date. As part of our ongoing search for active and possibly disease-relevant human endogenous retroviruses (HERVs), we have recently developed an oligonucleotide-based microarray. The assay allows for both the detection and the identification of most known retroviral reverse transcriptase (RT)-related nucleic acids in biological samples. In the present study, we have investigated the transcriptional activity of representative members of 20 HERV families in 19 different normal human tissues. Qualitative evaluation of chip hybridization signals and quantitative analysis by real-time RT-PCR revealed distinct HERV activity in the human tissues under investigation, suggesting that HERV elements are active in human cells in a tissue-specific manner. Most active members of HERV families were found in mRNA prepared from skin, thyroid gland, placenta, and tissues of reproductive organs. In contrast, only few active HERVs were detectable in muscle cells. Human tissues that lack HERV transcription could not be found, confirming that human endogenous retroviruses are permanent components of the human transcriptome. Distinct activity patterns may reflect the characteristics of the regulatory machinery in these cells, e.g., cell type-dependent occurrence of transcriptional regulatory factors.

Human endogenous retroviruses (HERVs) are normal components of the human genome. They are thought to be remnants of ancient germ line infections by exogenous retroviruses. Subsequent to germ line integration during the early radiation of primates, HERVs became subject to Mendelian inheritance like other cellular genes (for a review, see references 13, 15, 16, 41, 46, 47, 71, 72, and 87). During evolution, these mobile genetic elements have been amplified by repeated events of reintegration of reverse-transcribed mRNA (retrotransposition) and thus have spread throughout the genome (71, 72). Recent evaluation of the human genome sequencing data revealed that about 9% of the human genome is comprised of elements with long terminal repeats (LTRs) (LTR retrotransposons) (36, 43, 84) comprising over 200 families (30). The majority of these LTR elements, however, lack sequence similarity to retroviral genes within their internal region or constitute solitary LTRs. About 40 families identified so far have at least some members that show discernible homology to coding regions of retroviruses, but most of them have not yet been analyzed in depth (47, 74). These families are grouped into three classes based on the sequence homology of their *pol* regions with the *pol* genes of exogenous gammaretroviruses, betaretroviruses, and spumaviruses. They comprise around 200,000 entities (36), including about 230 full-length proviruses. Around 8,100 elements contain *pol*-related sequences, 3,661 of these with full or partial open reading frames (J. Blomberg, personal communication).

The vast majority of these HERV elements are presumably not infectious or functional, but there are numerous reports on the expression of endogenous retroviral transcripts in human tissues and cell lines, mostly of placental, embryonic, or neoplastic origin (2, 57, 81, 83; for a review, see references 18, 46, and 87). This suggests that a considerable number of HERV LTRs have retained their transcriptional activity.

Furthermore, there is evidence that transcription of at least some HERV families may be differentially regulated depending on the cell type (11, 51, 64, 85). Characterization of promoter activities of HERV-K, HERV-H, HERV-E, ERV9, and HERV-W families, the most intensively studied HERVs, revealed specific cell type preferences for each HERV family, and even individual elements of one family showed significant variation in transcription pattern (1, 7, 21, 23, 64, 70, 80). In some cases, transcription factor binding sites that interact with cell type-specific nuclear factors could be identified, demonstrating that the expression of HERVs is regulated in a complex and diverse manner comparable to cellular genes (19, 34, 35, 40, 54, 70; for a review, see reference 42).

A few HERV elements that possess intact open reading frames and the capability of encoding functional proteins have been reported. Pathogenic as well as nonpathogenic roles have been suggested for the corresponding gene products (for a

* Corresponding author. Mailing address: Medizinische Klinik, Universitätsklinikum Mannheim der Ruprecht-Karls-Universität Heidelberg, Wiesbadener Strasse 7-11, D-68305 Mannheim, Germany. Phone: 49 (0)621 383 4103. Fax: 49 (0)621 383 4201. E-mail: seifarth@rumms.uni-mannheim.de.

† W.S. and O.F. contributed equally.

review, see references 13, 44, 47, and 56). For example, some members of the HERV-K family encode retroviral enzymes with specific activities, such as protease (63), reverse transcriptase (RT) (8), integrase (32), and nonstructural proteins with functional similarities to human immunodeficiency virus (HIV) and human T-cell lymphotropic virus (HTLV) Rev and Rex proteins (14, 48, 49). Recently, a novel accessory gene, *np9*, that is located within the HERV-K *env* reading frame was found to be expressed in various human tumor tissues and transformed cell lines (4). An example of a HERV protein that serves an important function in human physiology is syncytin, which is encoded by a HERV-W *env* gene (12, 53). In this case, the fusogenic properties of a retroviral envelope protein are utilized for the formation of the placental syncytiotrophoblast layer.

There has also been much speculation that HERVs may contribute to some pathogenic conditions such as cancer, autoimmune diseases, and neurological disorders (for a review, see references 44, 58, 61, and 90). Increased transcript levels as well as *gag* and *env* gene products and corresponding antibodies against them have been observed in certain cancers. However, it is still unclear whether HERVs play a causative role, as is the case in some species such as mice and chicken. Increased HERV activity may as well represent a secondary effect of the pathogenic phenotype of the cell. The same holds true for the association of HERV elements with other human diseases. However, it is clear that viral proteins could be involved in autoimmunity triggering tolerance breakdown to autoantigens by mimicking the structure and function of cellular proteins.

An assessment of the numerous studies that associated HERV expression with human cancer is often hampered by the absence of comprehensive data on HERV activity in normal tissues. The majority of investigations have been performed with tumor-derived or in vitro-transformed cell lines. Thus, the transcriptional activity observed for specific HERV families may be influenced by chromosomal aberrations such as aneuploidy and alterations in chromatin configuration and methylation status characteristic for those cell lines and may not reflect the expression pattern of the corresponding primary tissues. Furthermore, previous studies were limited to only a few prominent HERVs for methodological reasons. As part of our ongoing search for disease-relevant HERV elements, we now present a systematic and comprehensive analysis of HERV transcription profiles in normal human tissues by using a retrovirus DNA chip (67). For our investigation, we selected 20 major families of class I, class II, and class III HERVs which have at least some full-length members (47, 74), and we report here the differential transcriptional regulation of these elements in a panel of 19 different postmortem human tissues.

MATERIALS AND METHODS

RNA samples. Total RNA from white blood cells and human brain tissue (prefrontal cortex; obtained from the Stanley Foundation Brain Collection, Bethesda, Md.) was extracted according to a guanidinium isothiocyanate-cesium chloride ultracentrifugation protocol (62). Total RNA from 17 different human tissues (Table 1) was purchased from Stratagene (La Jolla, Calif.) and from Ambion Inc. (Austin, Tex.). mRNA was prepared from total RNA samples by using Dynabead paramagnetic particles (Dynal, Hamburg, Germany) according to the manufacturer's protocol. To exclude genomic DNA contamination of the mRNA samples under investigation, 25 ng of each mRNA preparation was tested by PCR with the mixed oligonucleotide primers (MOPs) omitting the reverse transcription step (67). All samples ($n = 17$), except for two (blood and brain) prepared by using a guanidinium isothiocyanate-cesium chloride ultracentrifugation protocol, were DNA positive and were treated with 100 U of RNase-free DNase (Roche Molecular Biochemicals, Mannheim, Germany) in 100 mM sodium acetate (pH 5.0)–5 mM $MgSO_4$ until the repeated control PCR was negative. Only DNA-negative mRNA preparations were used in subsequent chip experiments.

TABLE 1. Origin and features of tissue RNA samples under investigation

Sample type[a]	Lot no.	No. of tissue samples	Sex of patient
PBMCs[b]		1	Male
Brain (prefrontal cortex)[c]		1	Male
Skeletal muscle	1100636	1	Female
Skin	0800181	1	Male
Heart	1000800	1	Male
Kidney	0900257	1	Female
Liver	1200114	1	Female
Lung	0800247	1	Male
Stomach	0900554	Pool of 3	Female
Colon	0800002	1	Female
Rectum	0600678	1	Male
Mammary gland	1200168	Pool of 7	Female
Ovary	1100642	1	Female
Uterus	1100640	Pool of 3	Female
Cervix	1200116	Pool of 2	Female
Placenta	0800315	1	Female
Testes	0620587	1	Male
Prostate[d]	013P010702028A	1	Male
Thyroid gland	0110350	Pool of 2	Female

[a] All samples were from Stratagene, except PBMCs, brain, and prostate.
[b] From a healthy volunteer.
[c] From the Stanley Foundation Brain Collection.
[d] From Ambion Inc.

Reverse transcription and PCR labeling. DNA-free mRNA (50 to 100 ng) was reverse transcribed in a volume of 50 μl containing a solution of 20 mM Tris-HCl (pH 8.4), 10 mM dithiothreitol, 50 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM each deoxynucleoside triphosphate, 10 U of RNasin (Promega), 30 pmol of random hexamer oligonucleotides (Promega), and 20 U of murine leukemia virus reverse transcriptase (GIBCO-BRL) at 37°C for 2 h. Subsequently, reverse-transcribed samples were denatured for 5 min at 95°C and stored at −20°C. Cy3-labeled DNA probes were synthesized by PCR using primer mixtures MOP-1 and MOP-2 as described previously (67). Amplified DNA fragments were purified by Biospin chromatography columns (Biogel P30; Bio-Rad Laboratories, Munich, Germany), ethanol precipitated, air dried, and redissolved in 25 μl of hybridization buffer containing 3.0× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5% sodium dodecyl sulfate, 50% Formamide, and 50 mM sodium phosphate buffer (pH 7.4).

Preparation and hybridization of microarrays. Preparation of microarrays and hybridization steps were carried out under standardized conditions as described previously (67). DNA chips of the same lot number, checked for quality consistency, were used for all experiments. Capture probes, including sequences corresponding to five housekeeping genes, are listed in Table 2. Sample treatment, PCR, and all chip hybridization reactions were performed in parallel. For data reliability, two replicas of the capture probe set were present on each DNA chip. All handling steps involving fluorochromes (Cy3 or Cy5) were performed in dimmed light, and incubations were carried out in the dark.

To determine the optimal hybridization conditions for efficient discrimination between different HERV families and subgroups of particular HERV families, pilot experiments were performed by using sets of HERV family-specific Cy3-labeled oligonucleotides for hybridization (data not shown). Under the stringency conditions employed, all HERV families could be reliably discriminated with these synthetic probes. Weak cross-hybridizations were observed only among the closely related members of HERV-K subgroups HML-2, HML-3, and HML-4 (67). Therefore, we arbitrarily defined a cutoff limit under standardized scanner settings which excluded signals of an intensity matching the cross-hybridization levels observed in the pilot experiments.

TABLE 2. Classification of retrovirus gene-specific capture probes used for microarrays[b]

Type of capture probe	Family group	RepBase name(s)[a]	Sequence source[c]	Dot code grid locus[d]
Class I retroviruses (gammaretrovirus-related elements)	HERV-I	HERVI	HERV-I (M92067) HERV-IP-T47D (U27241)	E9E10
		HERVIP10F	Seq65 (AP000842)	E11
	HERV-T	HERVS71	S71pCRTK6 (U12969) S71pCRTK1 (U12970)	F1 F2
	ERV-FRD	MER50I	ERV-FRD (U27240) Seq46 (S71606)	F3 F4
	HERV-E	HERVE	E4-1 (M10976) Seq32 (AC010636)	F5 F6
	HERV-H	HERVH	HERV-H (AF026252) Seq61 (AL359740) Seq66 (AL359740) RGH2 (D11078)	F7 F8 F9 F10
	HERV-F	HERVFH19I	HERV-Fb (AC000378)	F11
	HERV-W	HERV17	HERV-W (AF009668)	F12
	HERV-R	HERV3	ERV-3 (AC004609)	G12
	ERV9	HERV9	Seq64 (AC005253) Seq63 (AC018926) Seq60 (AL135749) Seq59 (AC006397) Seq49 (AF353709) ERV9 (X57147)	G6 G7 G8 G9 G10 G11
	Not assigned	Harlequin	Seq41 (AL158826)	G3
	Not assigned	MER21C	Seq36 (AC123767)	G4
Class II retroviruses (betaretrovirus-related elements)	HML-1	HERVK14I	HML-1 (U35102) Seq29 (S77579)	A8 A9
	HERV-K (HML-2)	HERVK	HERV-K10 (M14123) HERV clone M3.5 (U87592) HERV-K(D1.2) (U87595) HERV-K2.HOM (U87592) HERV-K(HP1) (U87588) HERV-K(P1.10) (U87594)	B1 B2 B3 B4 B5 B6
	HML-3	HERVK9I	HML-3 (U35236) HERV1 (S66676) RT244 (S77583) Seq26 (AC073115) Seq34 (AL592449) Seq42 (AF047595)	B7 B8 B9 B10 B11 B12
	HML-4	HERVK13I	Seq10 (AF047591) HERV-K-T47D (AF020092)	C10 C11
	HML-5	HERVK22I	HML-5 (U35161)	C9
	HERV-K (HML-6)	HERVK3I	HML-6 (U60269) Seq38 (AC010328) Seq56 (AC018558)	A10 A11 A12
	HML-8	HERVK11I	HERV-K(P3) (AF030047)	C5
	HERV-K (HML-10)	HERVKC4	Seq31 (AL162734) HERV-KC4 (U07856)	C7 C8

Continued on following page

TABLE 2—*Continued*

Type of capture probe	Family group	RepBase name(s)[a]	Sequence source[c]	Dot code grid locus[d]
Class III retroviruses (spumavirus-related elements)	HERV-L	HERVL-MLT2	HERV-L (X89211)	E2
			Seq39 (AC091914)	E3
			Seq40 (AC005076)	E4
			Seq45 (AC006971)	E5
			Seq48 (S71607)	E6
			Seq51 (AL353741)	E7
			Seq58 (AL590730)	E8
Human exogenous retroviruses			HIV-1 (K02013)	H2
			HIV-2 (J04542)	H3
			HTLV-1 (M81248)	H4
			HTLV-2 (M10060)	H5
			HFV (Y07725)	H7
Human housekeeping gene			Ubiquitin (U49869)	A2
			GAPDH (NM_002046.1)	A3
			Alpha-tubulin (NM_006082)	A4
			Beta-actin (E01094)	A5
			HPRT (NM_000194)	A6
Grid localization dots			Cy3-labeled oligonucleotide	A1, A7, D1, E1, E12, H1

[a] RepBase, Genetic Information Research Institute, Sunnyvale, Calif. (http://www.girinst.org) (30).
[b] Abbreviations: HML, human mouse mammary tumor like; HFV, human foamy virus.
[c] GenBank accession numbers are in parentheses.
[d] See Fig. 1 for grid.

Scanning and chip evaluation. After the hybridization and washing procedure, the dry glass slides were scanned with an Affymetrix (Santa Clara, Calif.) GMS 418 array scanner using the recommended settings for Cy3 fluorochrome. High-resolution images (10 μm/pixel) were saved in tagged image file format (16 bit) and bitmap file format (24 bit) and further processed with the ImaGene version 4.0 software tool package (BioDiscovery Inc., Los Angeles, Calif.). False-color mapping was used for result image visualization display. Further processing of digital images for array alignment (Fig. 1) was performed with Photoshop version 5.5 (Adobe Systems Inc.).

HERV taxon-specific primers for real-time PCR. HERV-specific primers for LightCycler real-time PCR were designed in such a way that for each HERV, one primer matched the capture probe sequences used in the corresponding microarray experiments. For the second primer, alignments of HERV reverse transcriptase sequences were conducted as described previously (66), and a consensus primer specific for representative members of a given HERV subfamily but with minimal homology to other HERV taxa was designed. This primer was located 100 to 150 bp upstream of the first primer. In the case of HERV-W, the second primer had to be placed downstream of the first primer to improve the specificity. Primer pairs sequences (in the 5′→3′ direction) used for eight representative HERV families and three housekeeping genes were as follows: for HERV-W, TGAGTCAATTCTCATACCTG (forward1) and AGTTAAGAGT TCTTGGGTGG (reverse1); for ERV-FRD AAAAAGGAAGAAGTTAA CAGC (forward) and ATATAAAGACTTAGGTCCTGC (reverse); for HERV-E, GGTGTCACTACTCAATACAC (forward) and GCAGCCTAGGTCT CTGG (reverse); for HERV-F, CCTCCAGTCACAACAACTC (forward1) and TATTGAAGAAGGCGGCTGG (reverse1); for HERV-K(HML-2), AAAGAA CCAGCCACCAGG (forward) and CAGTCTGAAAACTTTTCTCTC (reverse); for HERV-K(HML-3)-Seq26, CTGCAGCCTGCTAAGCG (forward) and CACTGTGAAAATTTTTTACGAG (reverse); for HERV-K(HML-5), TG AAAGGCCAGCTTGCTG (forward) and CAATTAGGAAATTCTTTTCTAC (reverse); for HERV-L, CTTCAGCTGGCAAGGCC (forward) and CCAGTG TGATATCTTGTGGC (reverse); for glucose 6-phosphate dehydrogenase (G6PD), TGCAGATGCTGTGTCTGG (forward) and CGTACTGGCCCAG GACC (reverse); for hypoxanthine phosphoribosyltransferase (HPRT), GTGA TGATGAACCAGGTTATGACCTTG (forward) and CTACAGTCATAGGA ATGGATCTATCAC (reverse); and for glycerlaldehyde-3-phosphate dehydrogenase (GAPDH), AGTCAACGGATTTGGTCGTATTGGG (forward) and ACGTACTCAGCGCCAGCATCG (reverse). HERV elements tested by real-time PCR are depicted in Fig. 1.

Real-time PCR and relative quantification of HERV expression. To determine the optimal LightCycler PCR parameters, several pilot experiments were performed according to established protocols (60, 69). Preliminary experiments were carried out with each single primer pair using dilutions of sample cDNAs to find primer annealing temperatures that yielded the greatest amount of specific product with melting temperatures separable from primer-dimer melting temperatures. The data acquisition temperature range of the LightCycler instrument was then adjusted to allow reliable discrimination between specific products and primer-dimer products. With these settings, negligible levels of unspecific PCR products were detectable in melting curve analysis and subsequent agarose gel electrophoresis (3% gel; 1× Tris-borate-EDTA [data not shown]) in only a few cases. To exclude PCR kinetic effects (due to measurements in the lag-plateau phase) influencing HERV-calibrator (HPRT and GAPDH [data not shown for G6PD]) measurements, cycle numbers were reduced from 45 to 30 cycles, warranting melting point analysis within the exponential range of both HERV and calibrator signal amplification. After optimization, the resulting PCR parameters were applied for subsequent LightCycler experiments.

Real-time PCR was carried out by using 5 μl of 1:100 cDNA sample dilutions and primers at 0.5 μmol in LightCycler FastStart DNA Master Plus SYBR Green I ready-to-use hot-start PCR mix (containing *Taq* DNA polymerase, reaction buffer, dUTP, and deoxyribonucleoside triphosphates dATP, dCTP, dGTP, the dye SYBR Green I, and $MgCl_2$; Roche Diagnostics GmbH). Cycling conditions for a Roche LightCycler instrument were as follows: an initial step of 95°C for 10 min and 30 cycles of 95°C for 1 s, 50°C for 5 s, and 72°C for 12 s. After 30 cycles, melting curves were generated for the final PCR products by decreasing the temperature to 50°C for 10 s followed by a increase in temperature to 95°C. Fluorescence was measured at 0.2°C increments. Relative quantification of HERV *pol* transcription was performed by using LightCycler software (version 3.5; Roche Molecular Biochemicals) and housekeeping genes as calibrators for normalization (77). Results expressed as the target-to-calibrator ratios of the

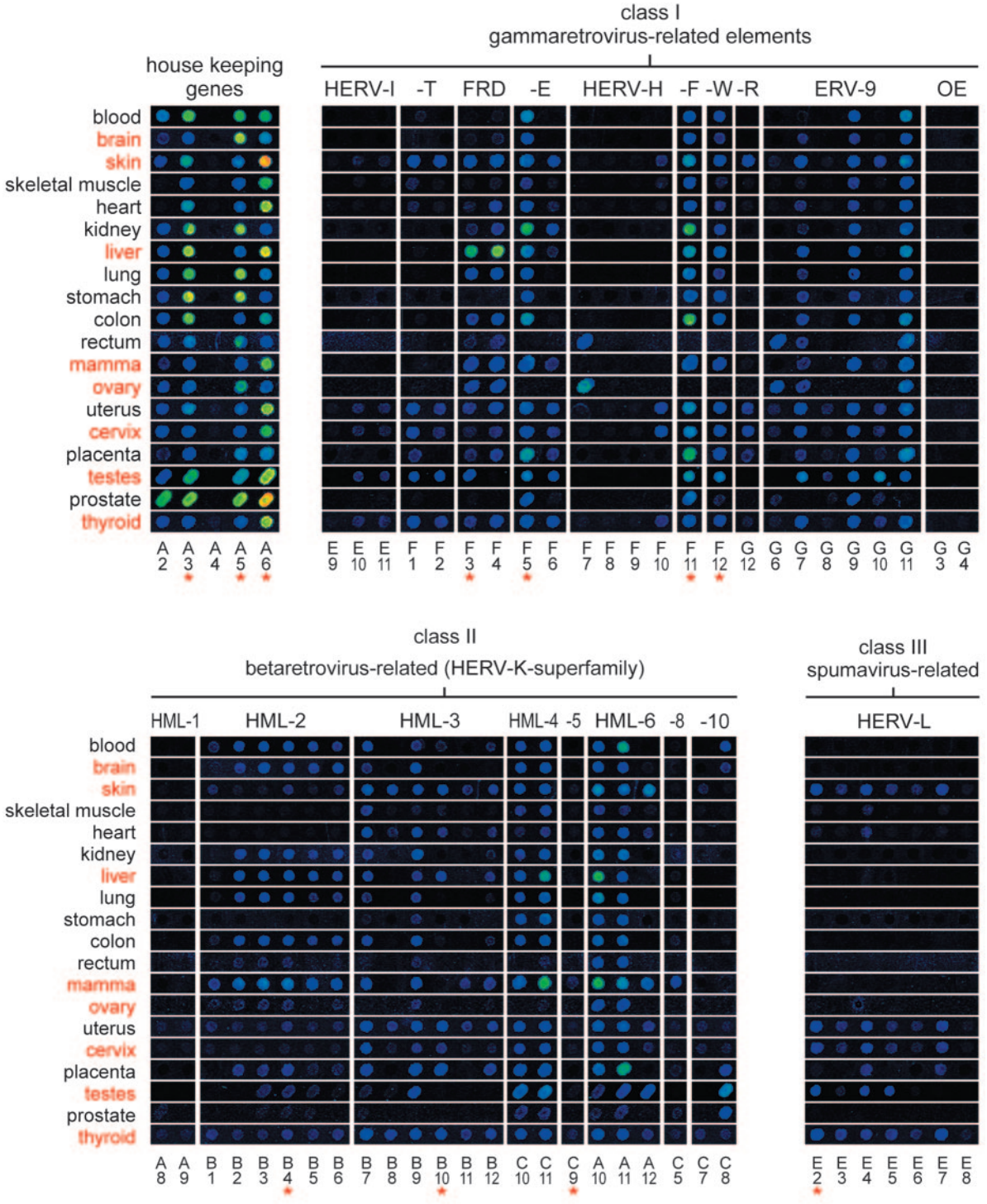

FIG. 1. Alignment of false-color chip data sets corresponding to HERV classes I, II, and III transcriptional activity observed in 19 normal human tissues by microarray hybridization. A housekeeping gene panel served as an internal control for mRNA integrity. DNA-free mRNA samples were analyzed by microarray hybridization after Cy3-labeled DNA hybridization probes had been generated by RT-PCR according to the standardized protocol. For data reliability, two replicas of the capture probe set were present on each DNA chip. Assays were carried out at least two to four times. For origins and identities of dots, see Table 2. Transcripts of exogenous retroviruses listed in Table 2 were not detected in any tissue. For validation and quantification, real-time PCR has been performed for a subset of eight tissues, denoted in red letters, and a subset of eight HERV elements, marked by red asterisks.

given samples were exported as tab-delimited text files and imported into Microsoft Excel 2000 software for further analysis.

RESULTS AND DISCUSSION

Microarray design and selection of HERV capture probes. A fast and highly sensitive DNA chip-based assay was used to investigate the transcriptional activity of human endogenous retroviruses in a panel of normal human tissues. The assay combines multiplex PCR using complex fluorochrome (Cy3)-modified primer cocktails and glass DNA chip hybridization allowing for direct analysis and comparison of HERV transcriptional profiles (67). Primer sequences were derived from two highly conserved amino acid motifs commonly found in all retroviral reverse transcriptase genes (65, 68). Discrimination between different HERV subgroups is achieved by the internal sequences of the amplification product that bind to specific oligonucleotides (capture probes) spotted on the chip. For capture probes, we selected 55 representative HERV sequences from 20 major HERV families which have at least a few full-length members (30, 47, 75) (Table 2). In addition, we included all human exogenous retroviruses known in the assay. Alignment of the sequences and phylogenetic analysis demonstrated that the different classes of sequences and many subgroup differences could be resolved (data not shown). This result further reinforces our confidence that the microarray is able to accurately distinguish specific HERV groups from one another. *pol* sequences of the most divergent members within a particular HERV family were used to enable detection of transcripts of the entire family. Family members with less than 20% sequence divergence were disregarded. An equivalent representation of each HERV family according to the total number of members was not possible due to the different degrees of sequence variation within one family. To measure the sequence variation within each family, different numbers of capture probes, for example, one for the HML-5 and seven for the HML-3 subgroups, were selected and spotted. Although HML-5 accounts for about 100 copies and HML-3 accounts for about 150 copies, HML-5 shows far less element variation. For the same reason, it is not possible to determine whether a particular hybridization signal is due to a single HERV element or is the result of a complex mixture of transcripts derived from several elements with *pol* sequences of more than 80% sequence identity. It might be expected, although it could not be tested, that within a multicopy HERV family comprising several subgroups, each with members of more than 80% sequence identity, hybridization signals would represent the mean transcriptional activity of all individual HERVs within one subgroup. Independent of the number of spotted family-specific capture probes, we defined a HERV family as active if at least one member or subgroup of this family gave a positive hybridization signal in repeated experiments (Table 3).

mRNA preparations from stomach, mammary gland, uterus, cervix, and thyroid gland were pooled from several individuals (Table 1), whereas samples from other tissues were derived from unique individuals. In previous experiments, HERV transcription activity in peripheral blood mononuclear cells (PBMCs) and mammary gland tissue samples from six different individuals were compared (67), and only slight variations in individual HERV transcription patterns were observed. Therefore, we assume that even mRNA samples taken from different unique individuals may be comparable with respect to tissue-specific transcription. Minor variation in the observed signal intensity or in number of hybridizing members of one HERV subgroup (Fig. 1) (67), however, may indeed be explained by different individual genetic backgrounds.

A digitally processed alignment of representative image data sets for all tissue specimens tested is shown in Fig. 1. Consistent signals obtained with capture probes for a panel of human housekeeping genes that serve as internal controls demonstrate the constant quality of the RNA samples and the reproducibility of the chip data. Retroviral activity profiles were constructed by qualitative evaluation of hybridization signals. The results of these comparative analyses are summarized in Table 3 in taxonomic order. For assignment of hybridization signals to HERV taxon and subgroup levels, see Table 2.

Differential transcriptional activity of HERV taxa. A characteristic expression activity and specificity ranging from ubiquitous and frequent to rare expression is observed for the different HERV taxa (Fig. 1). In general, class I and class II HERVs appear to be more active than the spumavirus-related class III elements. This correlates approximately with age and proximity to genes of these classes (37). Class I and class II HERVs both arose about 40 to 50 million years ago and repeatedly amplified during primate evolution. Some class II HERVs have still been actively transposing since the divergence of humans and chimpanzees less than 5 million years ago (5, 6). The older class III ERVs, however, can be traced back at least 70 million years and are generally underrepresented in gene-rich regions. One explanation for this unequal distribution is that these older elements have been successively eliminated from gene-rich regions throughout evolution, probably because of their potential to affect gene transcription (37). Our data support the hypothesis that most of the remaining copies have been silenced during evolution.

Among class I HERVs, the most abundantly expressed HERVs are the ERV9 elements which are transcribed in all tissues tested; the HERV-E, HERV-F, and HERV-W families, which were found to be active in all tissues with the exception of the rectum and ovary; and the HERV-FRD family, which is inactive only in PBMCs, stomach, and prostate. Transcripts of the high-copy-number HERV-H family were detected only in rectum, ovary, uterus, and cervix and to a lesser extent in skeletal muscle, skin, and thyroid gland. This finding contrasts with previous findings that suggested that HERV-H promoters are active in many different cell types (64, 85). However, this could be explained by the fact that about 90% of HERV-H elements are truncated in the *pol* gene and lack the sequences used as capture probes for our retrovirus chip (86). Therefore, the majority of HERV-H elements cannot be detected in this assay. Class I families, the transcriptional activity of which is restricted essentially to skin and reproductive tissues which are less frequently transcribed, are represented by the HERV-I and HERV-T groups. Transcripts of Harlequin and MER21C elements were not detected in any tissue.

Class II family transcripts were also detected in multiple tissues, indicating a widespread activity of these elements. Expression of HML-3 and HML-6 was found in all tissue samples. HML-4 members were also highly expressed in most tissues, with the exception of rectum and ovary tissues. HML-2 sequences, which represent the youngest HERV-K elements and which have retrotransposed in very recent evolutionary time (5,

TABLE 3. Transcriptional activity of HERV taxa in human tissues[a]

HERV	Sample type																		
	Blood	Brain	Skin	Skeletal muscle	Heart	Kidney	Liver	Lung	Stomach	Colon	Rectum	Mammary gland	Ovary	Uterus	Cervix	Placenta	Testes	Prostate	Thyroid gland
Class I																			
HERV-I	−	−	±	±	−	−	−	−	−	−	−	−	−	±	±	−	+	−	±
HERV-T	±	−	+	±	±	−	−	−	−	−	−	−	−	+	+	+	+	−	+
HERV-FRD	±	±	+	±	+	+	+	+	−	+	+	+	+	+	±	+	+	−	+
HERV-E	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+
HERV-H	−	−	+	±	±	−	−	−	−	−	+	−	+	+	+	−	−	−	+
HERV-F	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+
HERV-W	+	+	+	±	±	+	+	+	+	+	−	+	−	+	+	+	+	±	+
HERV-R	−	−	+	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	±
ERV9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harlequin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
MER21C	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Class II																			
HML-1	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	±	±
HML-2	+	+	±	−	−	+	+	+	−	+	±	+	+	±	±	+	+	±	+
HML-3	+	+	+	+	+	+	+	+	±	+	±	+	±	+	+	+	+	±	+
HML-4	+	+	+	+	+	+	+	+	+	+	±	+	±	+	+	+	+	+	+
HML-5	−	−	±	−	−	−	−	−	−	−	−	±	−	−	±	−	±	−	±
HML-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HML-8	−	−	−	−	−	±	−	−	−	−	−	+	−	±	−	−	−	±	±
HML-10	+	±	±	−	−	−	−	−	−	−	−	−	−	±	±	+	+	+	±
Class III																			
HERV-L	−	−	+	±	±	−	−	−	−	−	−	−	−	+	+	+	+	−	+

[a] HERV taxa were classified active if at least one member or subgroup of the taxon gave a positive hybridization signal in repeated experiments. +, clear signal; ±, weak signal; −, no signal.

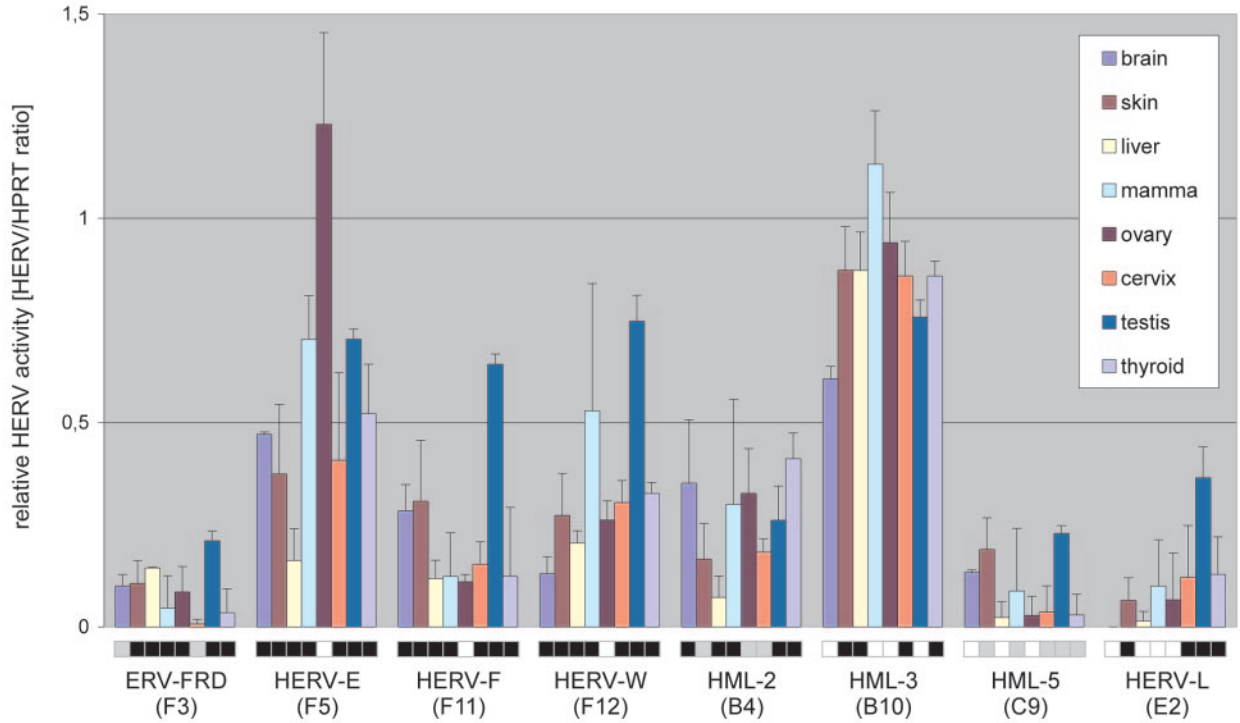

FIG. 2. Relative quantification of HERV transcriptional activity by real-time PCR. Transcriptional activity of eight HERV elements [ERV-FRD, HERV-E, HERV-F, HERV-W, HERV-K(HML-2), HERV-K(HML-3), HERV-K(HML-5), and HERV-L, all with dot localization codes shown in Fig. 1] was analyzed in eight different human tissues (brain, skin, liver, mammary gland, ovary, cervix, testes, and thyroid gland). The relative abundance of HERV transcripts in each tissue normalized by HPRT levels is depicted by bars and standard deviations (triple experiments). The results of the corresponding chip hybridization experiments are symbolized by small boxes allowing discrimination between no (white boxes), weak (grey boxes), and strong (black boxes) signals.

6, 17, 52), were found to be less active than expected, especially in reproductive tissues. This family comprises the only HERVs that are nearly intact and have open reading frames for all essential retroviral genes (50). Furthermore, these HERVs have been shown to be involved in the production of retrovirus-like particles in human teratocarcinoma cell lines (45). For this reason, some efficient mechanisms for downregulation of these elements might exist in normal human tissues. This is in concordance with other reports that describe enhanced transcriptional activity of HML-2 elements in breast cancer and germ cell tumors compared to the corresponding normal tissues (26–28, 82, 83). mRNAs of the remaining class II families, HML-10, HML-8, HML-1, and HML-5, exhibited minimal expression in few of the tissues tested (Fig. 1 and Table 3). This result is in agreement with previous findings in which HML-1 and HML-5 elements showed weaker expression than HML-2 and HML-3 in human PBMCs (3).

The spumavirus-related class III elements (HERV-L) were found to be transcriptionally active to a lesser degree than class I and class II retroviruses. Transcripts were detected mainly in skin, uterus, cervix, placenta, testes, and thyroid gland, confirming results of transient transfection experiments with isolated HERV-L promoter sequences (64; S.Weinhardt, unpublished data). Human exogenous retroviruses HIV type 1 (HIV-1), HIV-2, HTLV type 1 (HTLV-1), HTLV-2, and human foamy viruses could not be detected in any of the analyzed tissues.

Quantification of HERV transcription by real-time RT-PCR. Real-time RT-PCR was performed to confirm the qualitative results obtained with the retrovirus-specific microarray and to determine the relative levels of HERV expression. To obtain comparable results, we selected the same region of the retroviral *pol* gene for real-time RT-PCR that was used for chip hybridization. The MOPs amplifying the chip hybridization probe, however, could not be adopted for the real-time RT-PCR. Since the MOP primers have to cover a broad spectrum of retroviral targets, their specificity must be low, and single HERV taxa cannot be distinguished in one amplification experiment. To obtain adequate selectivity for real-time RT-PCR, we designed primers annealing to the internal sequences that in half of their length match the microarray capture probes and are specific for a single HERV taxon. The second primers were derived from sequences 100 to 150 bp upstream or downstream and were located outside of the chip hybridization probe. To compare the abundance of HERV transcripts in different tissues, the level of HERV expression was normalized to HPRT levels. Comparable results were obtained when G6PD or GAPDH was used as a calibrator (data not shown). In this manner, the transcriptional activity of eight HERV taxa was determined in eight different normal human tissues (Fig. 2). The results are broadly consistent with those obtained by chip hybridization (Fig. 1 and Table 3). Among class I elements, HERV-E elements showed the highest level of expression, followed by HERV-W and HERV-F (Fig. 1).

The overall activity of ERV-FRD elements appears to be somewhat lower, as expected, but the relative differences of transcript levels in the different tissues corresponded well with the microarray signals. From class II HERVs, members of the HML-3 subfamily are highly transcribed in nearly every cell type. As suggested by the chip data, only low levels of HML-2 transcripts were detected in most tissues. The very low activity of HML-5 sequences is also consistent with the results of microarray analysis, as liver and ovary displayed the least transcriptional activity compared to all other tissues. Class III elements were found to be less abundant than class I and class II HERVs in accordance with the microarray, showing relatively high HERV-L activity only in reproductive tissues. However, the level of transcription was lower in skin tissue and higher in mammary gland than that expected from the chip data. Taken together, the data ascertained by real-time PCR generally matched the occurrence of signals on the microarray. Some discrepancies, e.g., the higher transcript levels of some HERV taxa detected by real time PCR, may be explained by the high specificity of the real-time PCR primers compared to that of the MOP primers. For generation of the microarray hybridization probe, a complex mixture of less-specific primers which simultaneously amplified all HERV transcripts present in a specific tissue sample was used. This process may lead to competition between single HERV sequences for the best-matching primers, which occur in limited amounts. Therefore, the amount of amplification of a single HERV type is influenced by the presence of competing HERV transcripts, with the consequence that some HERV transcripts may be underrepresented in the hybridization probe. In this context, a limiting factor could be the quality of mRNA preparation that appears to play a more critical role in the microarray analysis than in real-time PCR. Other factors that may cause discrepancies between microarray and real-time PCR data may be the unavoidable differences between the microarray probe location and the location of real-time PCR primers. The different dynamic ranges for low levels or very high levels of expression may also influence the results (22). Generally, a systematic bias of microarray measurements leading to a consistent underestimation of relative differences in mRNA expression compared to real-time PCR was observed (91).

Both methods, the retrovirus-specific microarray and real-time PCR, have a specific scope and fidelity and therefore cannot substitute for each other but will generate complementary information. The retrovirus-specific microarray serves as a very fast and reliable method to obtain an overview of overall HERV activity in a given cell type and provides a rough estimate of the transcription levels of different HERV families. Real-time PCR then allows for precise quantification of transcripts of a single HERV taxon.

Characteristic HERV expression profiles of different human tissues. All mRNA preparations under investigation exhibited complex HERV transcription patterns (Fig. 1 and 2 and Table 3). Human tissues that lacked HERV transcripts were not found, confirming that endogenous retroviruses are permanent components of the human transcriptome and are actively transcribed in various cell types. High activity of nearly all HERV families, including class III elements, was observed in thyroid gland, skin, reproductive organ tissues (uterus, cervix, and testes), and tissue of embryonic origin (placenta). In skin, uterus, and cervix, class I HERVs were found to be particularly active. Class II HERV activity was conspicuous in normal PBMCs, brain, and mammary gland. In most other tissues, transcripts of class I and class II elements showed about the same range of activity, whereas transcripts of class III elements were completely missing.

Elevated HERV transcription in reproductive tissues, sometimes associated with the production of retroviral particles, has been reported previously, and several biological implications have been postulated (for a review, see references 39, 73, 78, and 87). A high transcriptional activity and retrotransposition rate in germ line cells is a prerequisite for efficient amplification and subsequent genetic fixation of endogenous retroviruses in a species. Therefore, it is conceivable that there has been selection on HERVs for high activity in such tissues. Activation of HERV transcription may also occur during embryogenesis as reflected by the high expression activity of nearly all HERV families in the placenta. Differentiating tissues such as those of fetal origin are often less methylated at certain stages, and thus, HERV expression in embryonic tissue could be a by-product of lack of suppression. On the other hand, at least some HERVs, such as HERV-R (ERV-3), have been found to be differentially regulated and expressed in an organ-specific manner during spermatogenesis (38) and human embryogenesis (2).

One could postulate that HERV elements may have a beneficial role in human development. Since retroviral envelope proteins have fusogenic properties, it was assumed that HERV-encoded Env proteins may play a role in the formation of the syncytiotrophoblast layer of the placenta (25). Several HERV families, HERV-T, HERV-FRD, HERV-F, HERV-W, HERV-R (ERV3) and HERV-K(HML-2), which are active in human placenta (Fig. 1) code for intact retroviral Env proteins (20). The hypothesis that Env proteins are involved in syncytiotrophoblast formation has been recently confirmed by the observation that the envelope proteins of HERV-W, now called syncytin-1 (12, 53), and of HERV-FRD, syncytin-2 (10), show fusogenic properties in a variety of mammalian cell lines. Inhibition of HERV-W Env protein expression leads to a decrease of trophoblast fusion and differentiation (24). Dysregulation of syncytin-1, therefore, contributes to altered cell fusion processes in placentogenesis and may play a role in hypertensive disorders of pregnancy such as preeclampsia and HELLP syndrome (33).

Another postulated beneficial role of HERV-encoded Env proteins is their involvement in suppressing possible maternal immunological reactions against the fetus based on the immunosuppressive activity characteristic of retroviral Env proteins (79, 87). Furthermore, expressed HERVs may block infectious retroviruses, thus protecting the growing fetus from exogenous retroviruses that could possibly be transferred from the maternal blood (39). In mice, at least two suppressor genes derived from endogenous retroviruses are known to prevent virus spread through receptor interference or inhibition of proviral integration (for a review, see reference 9). There is no direct evidence for such a protective mechanism in humans, but it is conspicuous that no infectious human exogenous retrovirus with significant similarity to a HERV has been detected so far. Recently, induction of cellular resistance against infection with spleen necrosis virus was demonstrated in canine cells

transfected with a HERV-W *env*-expressing plasmid (59). To achieve complete protection, expression of a wide variety of HERV families in the placenta would be theoretically favorable and is what we observed with our microarray.

A similarly high transcription level of nearly all HERV families was found in skin tissue (Fig. 1 and Table 3). This tissue tropism may also function as a defense mechanism against possible exogenous retroviruses. Furthermore, skin is normally exposed to many agents that may influence retroviral expression. For example, it was previously shown that UV radiation significantly activates transcription of a variety of HERVs in primary epidermal keratinocytes and in a spontaneously immortalized keratinocyte cell line (29).

In the mammary gland, class II HERV elements (subgroups HML-2, HML-3, HML-4, HML-5, HML-6, and HML-8) are predominantly expressed. This result is in concordance with several studies that reported a preferential expression of HML elements in this tissue (65, 88, 89). Steroid hormone-responsive elements in the LTRs, particularly the HML-2 subgroup, may explain the high activity of class II elements (34, 55). This could also explain the high level of HERV transcripts in the thyroid gland, where the largest number of HERV families is expressed. These HERVs may be activated through the thyroid hormone receptor since both thyroid and steroid hormone receptors belong to the same nuclear receptor superfamily and may recognize related sequences (76).

In contrast, less HERV activity was observed in rectum, stomach, heart, and skeletal muscle. Muscle tissues represent highly differentiated nondividing multinucleate cells with highly specialized tasks. This contrasts with tissues that have high rates of proliferation (uterus, placenta, and dermal cells) and metabolism (fetal cells) and with tissues with exocrine and endocrine activity (mammary and thyroid glands). Therefore, HERV activity may reflect the level of transcriptional and proliferative activity in situ.

Our data reveal that the activity of endogenous retroviruses is regulated differentially and is cell type specific, similar to normal gene regulation. The target site of integration may play a major role in determining whether a HERV sequence is placed in a transcriptionally active genomic locus. Quantification of some selected HERV taxa by real-time RT-PCR suggests that transcriptional activity roughly correlates with element copy numbers in the genome, insofar as elements with more than 150 copies [HERV-E(HML-3)] show higher transcript levels than elements in the range of 10 to 50 copies [ERV-FRD, HERV-F, and HERV-W(HML-2)]. In contrast, HERV-L elements comprising about 200 copies appear to be generally downregulated in most tissues. This could be due to underrepresention of these elements in gene-rich and transcriptionally active regions (37). Many HERVs appear to have been silenced by cellular mechanisms such as methylation. A possible role of methylation in differential activity of a subgroup of human-specific HERV-K(HML-2) LTRs, for example, is suggested by the differential methylation pattern of the corresponding loci in brain- and lymph node-derived genomic DNA (31). A further major regulative mechanism that influences the transcriptional activities and tissue preferences of HERV families or individual HERV elements is interaction with cellular transcription factors that recognize specific binding sites located within the retroviral LTRs. In previous studies and the present study, it has been shown that LTRs of HERV-H, HERV-W, HERV-K(HML-4), and HERV-L families may retain their cell type specificity independently from the genomic location when they are cloned in expression vectors and transiently transfected into different human cell lines and that they specifically bind to various cellular transcription factors (7, 64; Weinhardt, unpublished). Taken together, our findings suggest that HERVs behave like normal cellular genes and are a permanent component of the transcriptome of a cell. The data presented here offer a basis for further studies of HERV expression and regulation under physiological and pathological conditions.

ACKNOWLEDGMENTS

This work was supported by the Forschungsfonds of the Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim, Germany.

Postmortem brain tissue was donated by the Stanley Foundation Brain Collection courtesy of Michael B. Knable, E. Fuller Torrey, Maree J. Webster, and Robert H. Yolken.

REFERENCES

1. **Anderssen, S., E. Sjottem, G. Svineng, and T. Johansen.** 1997. Comparative analyses of LTRs of the ERV-H family of primate-specific retrovirus-like elements isolated from marmoset, African green monkey, and man. Virology **234:**14–30.
2. **Andersson, A. C., P. J. Venables, R. R. Tonjes, J. Scherer, L. Eriksson, and E. Larsson.** 2002. Developmental expression of HERV-R (ERV3) and HERV-K in human tissue. Virology **297:**220–225.
3. **Andersson, M. L., P. Medstrand, H. Yin, and J. Blomberg.** 1996. Differential expression of human endogenous retroviral sequences similar to mouse mammary tumor virus in normal peripheral blood mononuclear cells. AIDS Res. Hum. Retrovir. **12:**833–840.
4. **Armbruester, V., M. Sauter, E. Krautkraemer, E. Meese, A. Kleiman, B. Best, K. Roemer, and N. Mueller-Lantzsch.** 2002. A novel gene from the human endogenous retrovirus K expressed in transformed cells. Clin. Cancer Res. **8:**1800–1807.
5. **Barbulescu, M., G. Turner, M. I. Seaman, A. S. Deinard, K. K. Kidd, and J. Lenz.** 1999. Many human endogenous retrovirus K (HERV-K) proviruses are unique to humans. Curr. Biol. **9:**861–868.
6. **Barbulescu, M., G. Turner, M. Su, R. Kim, M. I. Jensen-Seaman, A. S. Deinard, K. K. Kidd, and J. Lenz.** 2001. A HERV-K provirus in chimpanzees, bonobos and gorillas, but not humans. Curr. Biol. **11:**779–783.
7. **Baust, C., W. Seifarth, U. Schon, R. Hehlmann, and C. Leib-Mosch.** 2001. Functional activity of HERV-K-T47D-related long terminal repeats. Virology **283:**262–272.
8. **Berkhout, B., M. Jebbink, and J. Zsiros.** 1999. Identification of an active reverse transcriptase enzyme encoded by a human endogenous HERV-K retrovirus. J. Virol. **73:**2365–2375.
9. **Best, S., P. R. Le Tissier, and J. P. Stoye.** 1997. Endogenous retroviruses and the evolution of resistance to retroviral infection. Trends Microbiol. **5:**313–318.
10. **Blaise, S., N. de Parseval, L. Benit, and T. Heidmann.** 2003. Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies syncytin 2, a gene conserved on primate evolution. Proc. Natl. Acad. Sci. USA **100:**13013–13018.
11. **Blond, J. L., F. Beseme, L. Duret, O. Bouton, F. Bedin, H. Perron, B. Mandrand, and F. Mallet.** 1999. Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family. J. Virol. **73:**1175–1185.
12. **Blond, J. L., D. Lavillette, V. Cheynet, O. Bouton, G. Oriol, S. Chapel-Fernandes, B. Mandrand, F. Mallet, and F. L. Cosset.** 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.
13. **Bock, M., and J. P. Stoye.** 2000. Endogenous retroviruses and the human germline. Curr. Opin. Genet. Dev. **10:**651–655.
14. **Boese, A., M. Sauter, and N. Mueller-Lantzsch.** 2000. A Rev-like NES mediates cytoplasmic localization of HERV-K cORF. FEBS Lett. **468:**65–67.
15. **Bromham, L.** 2002. The human zoo: retroviruses in the human genome. Trends Ecol. Evol. **17:**91–97.
16. **Brosius, J.** 1999. Genomes were forged by massive bombardments with retroelements and retrosequences. Genetica **107:**209–238.
17. **Buzdin, A., S. Ustyugova, K. Khodosevich, I. Mamedov, Y. Lebedev, G. Hunsmann, and E. Sverdlov.** 2003. Human-specific subfamilies of HERV-K

(HML-2) long terminal repeats: three master genes were active simultaneously during branching of hominoid lineages. Genomics **81:**149–156.
18. **Casau, A. E., J. E. Vaughan, G. Lozano, and A. J. Levine.** 1999. Germ cell expression of an isolated human endogenous retroviral long terminal repeat of the HERV-K/HTDV family in transgenic mice. J. Virol. **73:**9976–9983.
19. **de Parseval, N., H. Alkabbani, and T. Heidmann.** 1999. The long terminal repeats of the HERV-H human endogenous retrovirus contain binding sites for transcriptional regulation by the Myb protein. J. Gen. Virol. **80:**841–845.
20. **de Parseval, N., V. Lazar, J. F. Casella, L. Benit, and T. Heidmann.** 2003. Survey of human genes of retroviral origin: identification and transcriptome of the genes with coding capacity for complete envelope proteins. J. Virol. **77:**10414–10422.
21. **Domansky, A. N., E. P. Kopantzev, E. V. Snezhkov, Y. B. Lebedev, C. Leib-Mosch, and E. D. Sverdlov.** 2000. Solitary HERV-K LTRs possess bi-directional promoter activity and contain a negative regulatory element in the U5 region. FEBS Lett. **472:**191–195.
22. **Etienne, W., M. H. Meyer, J. Peppers, and R. A. Meyer, Jr.** 2004. Comparison of mRNA gene expression by RT-PCR and DNA microarray. BioTechniques **36:**618–620
23. **Feuchter, A., and D. Mager.** 1990. Functional heterogeneity of a large family of human LTR-like promoters and enhancers. Nucleic Acids Res. **18:**1261–1270.
24. **Frendo, J. L., D. Olivier, V. Cheynet, J. L. Blond, O. Bouton, M. Vidaud, M. Rabreau, D. Evain-Brion, and F. Mallet.** 2003. Direct involvement of HERV-W Env glycoprotein in human trophoblast cell fusion and differentiation. Mol. Cell. Biol. **23:**3566–3574.
25. **Harris, J. R.** 1991. The evolution of placental mammals. FEBS Lett. **295:**3–4.
26. **Herbst, H., M. Sauter, H. Fuchs, C. Kuhler-Obbarius, T. Loning, and N. Mueller-Lantzsch.** 1997. Gene products of human endogenous retrovirus (HERV)-K in germ cell and trophoblastic tumor. Verh. Dtsch. Ges. Pathol. **81:**464–470.
27. **Herbst, H., M. Sauter, C. Kuhler-Obbarius, T. Loning, and N. Mueller-Lantzsch.** 1998. Human endogenous retrovirus (HERV)-K transcripts in germ cell and trophoblastic tumours. APMIS **106:**216–220.
28. **Herbst, H., M. Sauter, and N. Mueller-Lantzsch.** 1996. Expression of human endogenous retrovirus K elements in germ cell and trophoblastic tumors. Am. J. Pathol. **149:**1727–1735.
29. **Hohenadl, C., H. Germaier, M. Walchner, M. Hagenhofer, M. Herrmann, M. Sturzl, P. Kind, R. Hehlmann, V. Erfle, and C. Leib-Mosch.** 1999. Transcriptional activation of endogenous retroviral sequences in human epidermal keratinocytes by UVB irradiation. J. Investig. Dermatol. **113:**587–594.
30. **Jurka, J.** 2000. Repbase update: a database and an electronic journal of repetitive elements. Trends Genet. **16:**418–420.
31. **Khodosevich, K., Y. Lebedev, and E. D. Sverdlov.** 2004. Large-scale determination of the methylation status of retrotransposons in different tissues using a methylation tags approach. Nucleic Acids Res. **32:**e31.
32. **Kitamura, Y., T. Ayukawa, T. Ishikawa, T. Kanda, and K. Yoshiike.** 1996. Human endogenous retrovirus K10 encodes a functional integrase. J. Virol. **70:**3302–3306.
33. **Knerr, I., E. Beinder, and W. Rascher.** 2002. Syncytin, a novel human endogenous retroviral gene in human placenta: evidence for its dysregulation in preeclampsia and HELLP syndrome. Am. J. Obstet. Gynecol. **186:**210–213.
34. **Knossl, M., R. Lower, and J. Lower.** 1999. Expression of the human endogenous retrovirus HTDV/HERV-K is enhanced by cellular transcription factor YY1. J. Virol. **73:**1254–1261.
35. **La Mantia, G., B. Majello, A. Di Cristofano, M. Strazzullo, G. Minchiotti, and L. Lania.** 1992. Identification of regulatory elements within the minimal promoter region of the human endogenous ERV9 proviruses: accurate transcription initiation is controlled by an Inr-like element. Nucleic Acids Res. **20:**4129–4136.
36. **Lander, E. S., L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. LeVine, P. McEwan, K. McKernan, J. Meldrim, J. P. Mesirov, C. Miranda, W. Morris, J. Naylor, C. Raymond, M. Rosetti, R. Santos, A. Sheridan, C. Sougnez, N. Stange-Thomann, N. Stojanovic, A. Subramanian, D. Wyman, J. Rogers, J. Sulston, R. Ainscough, S. Beck, D. Bentley, J. Burton, C. Clee, N. Carter, A. Coulson, R. Deadman, P. Deloukas, A. Dunham, I. Dunham, R. Durbin, L. French, D. Grafham, S. Gregory, T. Hubbard, S. Humphray, A. Hunt, M. Jones, C. Lloyd, A. McMurray, L. Matthews, S. Mercer, S. Milne, J. C. Mullikin, A. Mungall, R. Plumb, M. Ross, R. Shownkeen, S. Sims, R. H. Waterston, R. K. Wilson, L. W. Hillier, J. D. McPherson, M. A. Marra, E. R. Mardis, L. A. Fulton, A. T. Chinwalla, K. H. Pepin, W. R. Gish, S. L. Chissoe, M. C. Wendl, K. D. Delehaunty, T. L. Miner, A. Delehaunty, J. B. Kramer, L. L. Cook, R. S. Fulton, D. L. Johnson, P. J. Minx, S. W. Clifton, T. Hawkins, E. Branscomb, P. Predki, P. Richardson, S. Wenning, T. Slezak, N. Doggett, J. F. Cheng, A. Olsen, S. Lucas, C. Elkin, E. Uberbacher, M. Frazier, et al.** 2001. Initial sequencing and analysis of the human genome. Nature **409:**860–921.
37. **Landry, J. R., A. Rouhi, P. Medstrand, and D. L. Mager.** 2002. The Opitz syndrome gene *Mid1* is transcribed from a human endogenous retroviral promoter. Mol. Biol. Evol. **19:**1934–1942.
38. **Larsson, E., A. C. Andersson, and B. O. Nilsson.** 1994. Expression of an endogenous retrovirus (ERV3 HERV-R) in human reproductive and embryonic tissues—evidence for a function for envelope gene products. Ups. J. Med. Sci. **99:**113–120.
39. **Larsson, E., and G. Andersson.** 1998. Beneficial role of human endogenous retroviruses: facts and hypotheses. Scand. J. Immunol. **48:**329–338.
40. **Lee, W. J., H. J. Kwun, and K. L. Jang.** 2003. Analysis of transcriptional regulatory sequences in the human endogenous retrovirus W long terminal repeat. J. Gen. Virol. **84:**2229–2235.
41. **Leib-Mösch, C., and W. Seifarth.** 1996. Evolution and biological significance of human retroelements. Virus Genes **11:**133–145.
42. **Leib-Mösch, C., W. Seifarth, and U. Schön.** 2004. Influence of human endogenous retroviruses on cellular gene expression, p. 124–145. *In* E. Sverdlov (ed.), Retroviruses and primate genome evolution. Landes Bioscience, Georgetown, Texas.
43. **Li, W. H., Z. Gu, H. Wang, and A. Nekrutenko.** 2001. Evolutionary analyses of the human genome. Nature **409:**847-849.
44. **Löwer, R.** 1999. The pathogenic potential of endogenous retroviruses: facts and fantasies. Trends Microbiol. **7:**350-356.
45. **Löwer, R., K. Boller, B. Hasenmaier, C. Korbmacher, N. Muller-Lantzsch, J. Löwer, and R. Kurth.** 1993. Identification of human endogenous retroviruses with complex mRNA expression and particle formation. Proc. Natl. Acad. Sci. USA **90:**4480-4484.
46. **Löwer, R., J. Löwer, and R. Kurth.** 1996. The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. Proc. Natl. Acad. Sci. USA **93:**5177-5184.
47. **Mager, D. L., and P. Medstrand.** 2003. Retroviral repeat sequences, p. 57–63. *In* D. Cooper (ed.), Nature encyclopedia of the human genome. Nature Publishing Group, London, United Kingdom.
48. **Magin, C., J. Hesse, J. Lower, and R. Lower.** 2000. Corf, the Rev/Rex homologue of HTDV/HERV-K, encodes an arginine-rich nuclear localization signal that exerts a *trans*-dominant phenotype when mutated. Virology **274:**11-16.
49. **Magin-Lachmann, C., S. Hahn, H. Strobel, U. Held, J. Lower, and R. Lower.** 2001. Rec (formerly Corf) function requires interaction with a complex, folded RNA structure within its responsive element rather than binding to a discrete specific binding site. J. Virol. **75:**10359-10371.
50. **Mayer, J., M. Sauter, A. Racz, D. Scherer, N. Mueller-Lantzsch, and E. Meese.** 1999. An almost-intact human endogenous retrovirus K on human chromosome 7. Nat. Genet. **21:**257-258.
51. **Medstrand, P., and J. Blomberg.** 1993. Characterization of novel reverse transcriptase encoding human endogenous retroviral sequences similar to type A and type B retroviruses: differential transcription in normal human tissues. J. Virol. **67:**6778-6787.
52. **Medstrand, P., and D. L. Mager.** 1998. Human-specific integrations of the HERV-K endogenous retrovirus family. J. Virol. **72:**9782-9787.
53. **Mi, S., X. Lee, X. Li, G. M. Veldman, H. Finnerty, L. Racie, E. LaVallie, X. Y. Tang, P. Edouard, S. Howes, J. C. Keith, Jr., and J. M. McCoy.** 2000. Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. Nature **403:**785-789.
54. **Nelson, D. T., N. L. Goodchild, and D. L. Mager.** 1996. Gain of Sp1 sites and loss of repressor sequences associated with a young, transcriptionally active subset of HERV-H endogenous long terminal repeats. Virology **220:**213-218.
55. **Ono, M., M. Kawakami, and H. Ushikubo.** 1987. Stimulation of expression of the human endogenous retrovirus genome by female steroid hormones in human breast cancer cell line T47D. J. Virol. **61:**2059-2062.
56. **Patience, C., D. A. Wilkinson, and R. A. Weiss.** 1997. Our retroviral heritage. Trends Genet. **13:**116-120.
57. **Patzke, S., M. Lindeskog, E. Munthe, and H. C. Aasheim.** 2002. Characterization of a novel human endogenous retrovirus, HERV-H/F, expressed in human leukemia cell lines. Virology **303:**164-173.
58. **Perl, A.** 2003. Role of endogenous retroviruses in autoimmune diseases. Rheum. Dis. Clin. N. Am. **29:**123-143.
59. **Ponferrada, V. G., B. S. Mauck, and D. P. Wooley.** 2003. The envelope glycoprotein of human endogenous retrovirus HERV-W induces cellular resistance to spleen necrosis virus. Arch. Virol. **148:**659-675.
60. **Rajeevan, M. S., S. D. Vernon, N. Taysavang, and E. R. Unger.** 2001. Validation of array-based gene expression profiles by real-time (kinetic) RT-PCR. J. Mol. Diagn. **3:**26-31.
61. **Rasmussen, H. B., G. Lucotte, and J. Clausen.** 2000. Endogenous retroviruses and multiple sclerosis. J. Neurovirol. **6**(Suppl. 2)**:**S80–S84.
62. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
63. **Schommer, S., M. Sauter, H. G. Krausslich, B. Best, and N. Mueller-Lantzsch.** 1996. Characterization of the human endogenous retrovirus K proteinase. J. Gen. Virol. **77:**375-379.
64. **Schön, U., W. Seifarth, C. Baust, C. Hohenadl, V. Erfle, and C. Leib-Mösch.** 2001. Cell type-specific expression and promoter activity of human endogenous retroviral long terminal repeats. Virology **279:**280-291.

65. **Seifarth, W., C. Baust, A. Murr, H. Skladny, F. Krieg-Schneider, J. Blusch, T. Werner, R. Hehlmann, and C. Leib-Mösch.** 1998. Proviral structure, chromosomal location, and expression of HERV-K-T47D, a novel human endogenous retrovirus derived from T47D particles. J. Virol. **72:**8384-8391.
66. **Seifarth, W., U. Krause, C. Hohenadl, C. Baust, R. Hehlmann, and C. Leib-Mösch.** 2000. Rapid identification of all known retroviral reverse transcriptase sequences with a novel versatile detection assay. AIDS Res. Hum. Retrovir. **16:**721-729.
67. **Seifarth, W., B. Spiess, U. Zeilfelder, C. Speth, R. Hehlmann, and C. Leib-Mösch.** 2003. Assessment of retroviral activity using a universal retrovirus chip. J. Virol. Methods **112:**79-91.
68. **Shih, A., R. Misra, and M. G. Rush.** 1989. Detection of multiple, novel reverse transcriptase coding sequences in human nucleic acids: relation to primate retroviruses. J. Virol. **63:**64-75.
69. **Simpson, D. A., S. Feeney, C. Boyle, and A. W. Stitt.** 2000. Retinal VEGF mRNA measured by SYBR Green I fluorescence: a versatile approach to quantitative PCR. Mol. Vis. **6:**178-183.
70. **Sjottem, E., S. Anderssen, and T. Johansen.** 1996. The promoter activity of long terminal repeats of the HERV-H family of human retrovirus-like elements is critically dependent on Sp1 family proteins interacting with a GC/GT box located immediately 3′ to the TATA box. J. Virol. **70:**188-198.
71. **Sverdlov, E.** 2003. Retroviruses and primate genome evolution. Landes Bioscience, Georgetown, Texas.
72. **Sverdlov, E. D.** 2000. Retroviruses and primate evolution. Bioessays **22:**161-171.
73. **Taruscio, D., and A. Mantovani.** 1998. Human endogenous retroviral sequences: possible roles in reproductive physiopathology. Biol. Reprod. **59:** 713-724.
74. **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.
75. **Tristem, M., T. Myles, and F. Hill.** 1995. A highly divergent retroviral sequence in the tuatara (Sphenodon). Virology **210:**206-211.
76. **Umesono, K., and R. M. Evans.** 1989. Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell **57:**1139-1146.
77. **Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman.** 18 June 2002, posting date. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. *3:*RESEARCH0034. [Online.] http://genomebiology.com/2002/3/7/research/0034.
78. **Venables, P. J., S. M. Brookes, D. Griffiths, R. A. Weiss, and M. T. Boyd.** 1995. Abundance of an endogenous retroviral envelope protein in placental trophoblasts suggests a biological function. Virology **211:**589-592.
79. **Villareal, L. P.** 1997. On viruses, sex, and motherhood. J. Virol. **71:**859-865.
80. **Vinogradova, T. V., L. P. Leppik, L. G. Nikolaev, S. B. Akopov, A. M. Kleiman, N. B. Senyuta, and E. D. Sverdlov.** 2001. Solitary human endogenous retroviruses-K LTRs retain transcriptional activity in vivo, the mode of which is different in different cell types. Virology **290:**83-90.
81. **Wang-Johanning, F., A. R. Frost, B. Jian, R. Azerou, D. W. Lu, D. T. Chen, and G. L. Johanning.** 2003. Detecting the expression of human endogenous retrovirus E envelope transcripts in human prostate adenocarcinoma. Cancer **98:**187-197.
82. **Wang-Johanning, F., A. R. Frost, B. Jian, L. Epp, D. W. Lu, and G. L. Johanning.** 2003. Quantitation of HERV-K *env* gene expression and splicing in human breast cancer. Oncogene **22:**1528-1535.
83. **Wang-Johanning, F., A. R. Frost, G. L. Johanning, M. B. Khazaeli, A. F. LoBuglio, D. R. Shaw, and T. V. Strong.** 2001. Expression of human endogenous retrovirus K envelope transcripts in human breast cancer. Clin. Cancer Res. **7:**1553-1560.
84. **Waterston, R. H., K. Lindblad-Toh, E. Birney, J. Rogers, J. F. Abril, P. Agarwal, R. Agarwala, R. Ainscough, M. Alexandersson, P. An, S. E. Antonarakis, J. Attwood, R. Baertsch, J. Bailey, K. Barlow, S. Beck, E. Berry, B. Birren, T. Bloom, P. Bork, M. Botcherby, N. Bray, M. R. Brent, D. G. Brown, S. D. Brown, C. Bult, J. Burton, J. Butler, R. D. Campbell, P. Carninci, S. Cawley, F. Chiaromonte, A. T. Chinwalla, D. M. Church, M. Clamp, C. Clee, F. S. Collins, L. L. Cook, R. R. Copley, A. Coulson, O. Couronne, J. Cuff, V. Curwen, T. Cutts, M. Daly, R. David, J. Davies, K. D. Delehaunty, J. Deri, E. T. Dermitzakis, C. Dewey, N. J. Dickens, M. Diekhans, S. Dodge, I. Dubchak, D. M. Dunn, S. R. Eddy, L. Elnitski, R. D. Emes, P. Eswara, E. Eyras, A. Felsenfeld, G. A. Fewell, P. Flicek, K. Foley, W. N. Frankel, L. A. Fulton, R. S. Fulton, T. S. Furey, D. Gage, R. A. Gibbs, G. Glusman, S. Gnerre, N. Goldman, L. Goodstadt, D. Grafham, T. A. Graves, E. D. Green, S. Gregory, R. Guigo, M. Guyer, R. C. Hardison, D. Haussler, Y. Hayashizaki, L. W. Hillier, A. Hinrichs, W. Hlavina, T. Holzer, F. Hsu, A. Hua, T. Hubbard, A. Hunt, I. Jackson, D. B. Jaffe, L. S. Johnson, M. Jones, T. A. Jones, A. Joy, M. Kamal, E. K. Karlsson, et al.** 2002. Initial sequencing and comparative analysis of the mouse genome. Nature **420:**520-562.
85. **Wilkinson, D. A., J. D. Freeman, N. L. Goodchild, C. A. Kelleher, and D. L. Mager.** 1990. Autonomous expression of RTVL-H endogenous retrovirus-like elements in human cells. J. Virol. **64:**2157-2167.
86. **Wilkinson, D. A., N. L. Goodchild, T. M. Saxton, S. Wood, and D. L. Mager.** 1993. Evidence for a functional subclass of the RTVL-H family of human. J. Virol. **67:**2981-2989.
87. **Wilkinson, D. A., D. L. Mager, and J. C. Leong.** 1994. Endogenous human retroviruses, vol. 3. Plenum Press, New York, N.Y.
88. **Yin, H., P. Medstrand, M. L. Andersson, A. Borg, H. Olsson, and J. Blomberg.** 1997. Transcription of human endogenous retroviral sequences related to mouse mammary tumor virus in human breast and placenta: similar pattern in most malignant and nonmalignant breast tissues. AIDS Res. Hum. Retrovir. **13:**507-516.
89. **Yin, H., P. Medstrand, A. Kristofferson, U. Dietrich, P. Aman, and J. Blomberg.** 1999. Characterization of human MMTV-like (HML) elements similar to a sequence that was highly expressed in a human breast cancer: further definition of the HML-6 group. Virology **256:**22-35.
90. **Yolken, R. H., and E. F. Torrey.** 1995. Viruses, schizophrenia, and bipolar disorder. Clin. Microbiol. Rev. **8:**131-145.
91. **Yuen, T., E. Wurmbach, R. L. Pfeffer, B. J. Ebersole, and S. C. Sealfon.** 2002. Accuracy and calibration of commercial oligonucleotide and custom cDNA microarray. Nucleic Acids Res. **30:**e48.