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Review

The viruses in all of us: Characteristics and biological significance of human endogenous retrovirus sequences

(reverse transcriptase/retroelements/human teratocarcinoma-derived virus)

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ABSTRACT **Human endogenous retroviruses (HERVs) are very likely footprints of ancient germ-cell infections. HERV sequences encompass about 1% of the human genome. HERVs have retained the potential of other retroelements to retrotranspose and thus to change genomic structure and function. The genomes of almost all HERV families are highly defective. Recent progress has allowed the identification of the biologically most active family, HTDV/HERV-K, which codes for viral proteins and particles and is highly expressed in germ-cell tumors. The demonstrable and potential roles of HTDV/HERV-K as well as of other human elements in disease and in maintaining genome plasticity are illustrated.**

All human beings carry *h*uman *e*ndogenous *r*etro *v*irus (HERV) sequences as an integral part of their genomes. In contrast, exogenous retrovirus strains occur only in those cells of an infected individual which support virus entry and replication. It is usually assumed that at some time during the course of human evolution, exogenous progenitors of HERVs have inserted themselves into the cells of the germ line, where they have been replicated along with the host's cellular genes. Furthermore, due to their unique genomic structure, HERVs have been subjected to many amplification and transposition events, resulting in a widespread distribution of complete or partial retroviral sequences throughout the human genome. Another working hypothesis, put forward by Temin (1), postulates the consecutive evolution of complex retroelements from more simply structured ancestors.

Retroelements: From Reverse Transcriptase (RT) to Retroviruses

Endogenous retroviruses (ERVs) may exist as "endogenized" variants of exogenous virus strains. The mouse mammary tumor virus (MMTV) (2), and the Jaagsiekte sheep retrovirus (JSRV) (3), for example, are found as exogenous as well as endogenous agents in their host species. Alternatively, ERVs may have developed from ancestral retroelements (see Fig. 1). A prerequisite for the formation of retroelements is reverse transcription followed by retrotransposition. Transposed elements are flanked by short direct repeats of the target site which are created during the integration procedure.

Temin (1) favored the idea that retroelements have evolved along with an RT gene. This hypothetical scenario envisages a consecutive specialization of an ancestral RNA-dependent DNA polymerase, the RT predecessor. The composition of the different types of retroelements present in eukaryotes, including humans, reflects the acquisition of additional enzymatic activities (RNase H and Integrase domains, Protease; see Fig. 1), as well as the successive association with sequences exerting a regulatory potential (promoter) or with sequences coding for structural genes. Fig. 1 schematically illustrates the genome structure of these sequences and their hypothetical evolutionary relationship. Additional characteristics are depicted in Table 1. In Fig. 2, the putative life cycles of retroelements and the known life cycle of exogenous retroviruses are compared.

Pseudogenes are examples of rare chance reverse transcription and reintegration of cellular mRNA species (Fig. 1 and Fig. 2*A*). Pseudogenes which have acquired promoter sequences and thus are actively transcribed have been designated *retrogenes* (Table 1). Short interspersed elements (*SINE*s) may belong to the same category. However, in contrast to retrogenes, SINEs lack coding capacities. They are amplified to extremely high copy numbers (Table 1) and have been subjected to frequent point mutations and deletions. Two human families have been extensively studied, the *Alu* family (5) and the SINE-R family (6), which will be described in more detail below.

Prototype *retroposons* like the long interspersed elements (*LINE*s) (7), possess an internal G+C-rich promoter and a gene coding for an only partially characterized protein (ORF 1) in addition to a *pol* gene with RT homology (Fig. 1 and Table 1). Both gene products cofractionate with LINE mRNA in ribonucleoprotein particles (8, 9). Like SINEs, LINE families have been amplified to extremely high copy numbers (Table 1). However, most SINE and LINE elements contain multiple mutations and deletions, preferably in the 5′ region.

Retrotransposons evolved in a variety of organisms ranging from protozoa to human beings (10, 11). In these elements, RT genes are linked to genes that code for polyproteins with the potential to self-aggregate and to form core particles (Figs. 1 and 2). These proteins are the equivalents of the retroviral capsid proteins usually designated group-specific antigens (Gag). Retrotransposon RNA can be specifically incorporated into such particles, as it contains a packaging signal Ψ (psi). These retroelements are flanked by LTRs, which harbor promoter sequences (Figs. 1 and 2*B*). Retrotransposons are also amplified to high copy numbers (Table 1), and many of these elements are defective. Additionally, recombination between LTRs and excision of the internal sequences frequently results in the formation of *solitary LTRs*. Retrotransposons have been extensively studied in yeast, *Drosophila*, and mice. They may be either the derivatives or predecessors of retroviruses.

Retroviruses differ from retrotransposons by the presence of at least one additional coding region, the envelope (*env*) gene, which codes for viral membrane proteins. Retroviral *gag* gene products have acquired the ability to be transported to the cell surface and to bud from the cell membrane, incorporating Env

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Abbreviations: HERV, human endogenous retrovirus; ERV, endogenous retrovirus; MMTV, mouse mammary tumor virus; JSRV, Jaagsiekte sheep retrovirus; RT, reverse transcriptase; LTR, long terminal repeat; ORF, open reading frame; cORF, central ORF; SINE, short interspersed element; LINE, long interspersed element.

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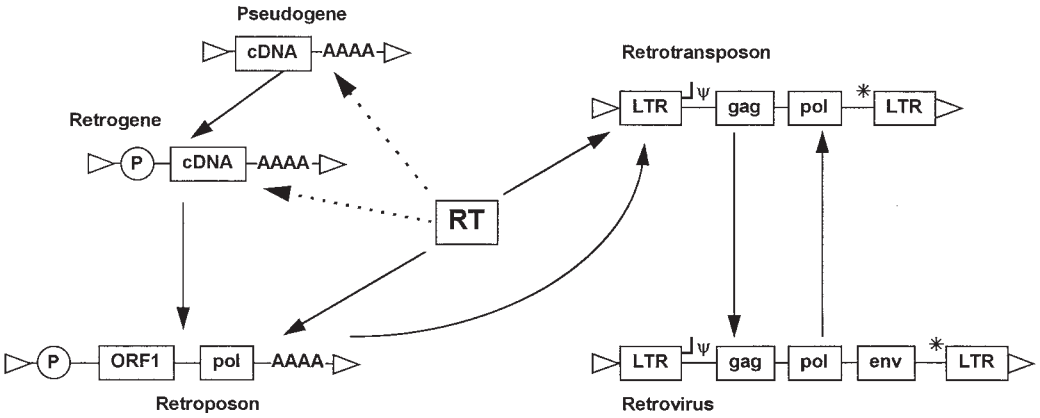

FIG. 1. Putative evolution of retroelements. Dotted arrows, generation of genetic elements by reverse transcription; solid arrows, acquisition of new elements by recombination; RT, reverse transcriptase gene; ORF, open reading frame; gag, capsid protein gene; pol, polymerase gene coding for RT, RNase H, integrase, and other enzymatic activities; env, envelope protein gene; LTR, long terminal repeat; P, promoter; AAAA, poly(A) tail; ▷, direct repeats (cellular DNA); ⌋, primer binding site; Ψ, packaging signal; *, polypurine tract.

proteins during this process (Figs. 1 and 2*C*). Env mediates the binding of virus particles to their cellular receptors, enabling virus entry, the first step in a new replication cycle (Fig. 2*D*). Thus, the *env* gene adds to retroelements the ability to spread between cells and individuals (infectivity).

The concerted action of Gag and Env proteins directing HERVs to budding and cellular export may exclude these sequences from the retrotransposition pathway. In addition, blocking of the receptor by secreted Env molecules may hinder re-infection of virus-producing cells (receptor interference) and re-integration of viral genomes. Both effects together could explain the limitations in copy number of HERV proviruses compared with other retroelements (Table 1). Proviruses of the HERV-H family, for instance, that have retained *env*-related sequences (12) are present in only 50 copies, whereas proviruses without the *env* gene exhibit a much higher amplification (see Table 1).

Therefore, at first sight, elements with retrotransposon structure seem to be more prone to amplification than retroviruses, reflecting perhaps their different routes of replication: intracellular versus intercellular spread. Surprisingly, however, a moderate copy number of full-length proviruses is always accompanied by a considerable frequency of solitary LTRs (Table 1), suggesting that HERVs may indeed spread very efficiently either by infection or by retrotransposition. The subsequent formation of solitary LTRs by excision of coding sequences may indicate that an unrestricted increase of retroelements with an RT gene and LTRs would be detrimental. Truncation of the promoter sequences at the 5′ ends of SINEs and LINEs may mirror a similar constraint. Alternatively, the latter may simply examplify insufficient reverse transcription starting from the 3′ end of transcripts.

Retroviruses may be seen as specialized mobile retroelements able to spread rapidly in a host population. Under the selective pressure of an extracellular exogenous life cycle, they take advantage of the plasticity inherent to RNA genomes. Endogenization of exogenous retroviruses can then be interpreted as an adaptation to the far slower evolutionary pace of the host.

Discovery of HERV Families

HERVs (see Table 2) have been discovered as a result of different experimental approaches. Screening human genomic libraries under low-stringency conditions with probes derived from animal retroviruses has allowed the isolation and characterization of multiple, albeit defective, proviruses, representing different families [e.g., HERV-E (17), HERV-R (13), HTDV/HERV-K (21)]. Other approaches relied on the use of oligonucleotides with homology to viral primer binding sites (HERV-P; ref. 20). Furthermore, HERV families were detected by chance during analyses of human gene loci [HERV-H (11), ERV-9 (14), HERV-I (18)]. Table 2 summarizes some of the characteristics of these HERV families.

HERVs have been classified according to their homologies to animal retroviruses (reviewed in ref. 23). Class I families have sequence similarities to mammalian type C retroviruses. Three families sharing substantial homologies not only in the well-conserved *pol* region but also in the *gag* and *env* genes have been grouped into a superfamily, the ERI family. Their closest infectious relatives are murine leukemia virus (MuLV) and baboon endogenous virus (BaEV). Other HERV families, such as ERV-9, HERV-I, and especially HERV-H, are more distantly related.

Class II families exhibit homology to mammalian type B and D retrovirus strains. Proviruses from the HTDV/HERV-K family (clone HERV-K 10; ref. 33) and from the HERV-K (C4) family (34) have been fully sequenced. Sharing homologies in the *gag*, *pol*, and *env* genes, they may also belong to a superfamily. Additional, less-characterized class II families exist. Cross-

Table 1. Characteristics of retroelements

Genomic structure	Designation	Example	Copy number	Refs.
Retroelement without RT, with cellular promoter	Retrogene (pseudogene)	Human phosphoglycerate kinase	10^0 to 10^1	4
Retroelement without RT, with internal promoter	SINE	*Alu*	10^5 to 10^6	5
		SINE-R	10^3	6
Retroelement with RT and internal promoter	Retroposon	LINE 1	10^4 to 10^5	7–9
Retroelement with RT and LTR	Retrotransposon	Ty*1* in *Saccharomyces cerevisiae*	10^2 to 10^4	10
		HERV-H with retroposon structure		11
Retroelement with RT, LTR, and *env*	Endogenous retrovirus	HERV-H, HERV-R, ERV-9, HTDV/HERV-K	10^0 to 10^2	12–15
LTR	Solitary LTR	HTDV/HERV-K	10^1 to 10^4	16

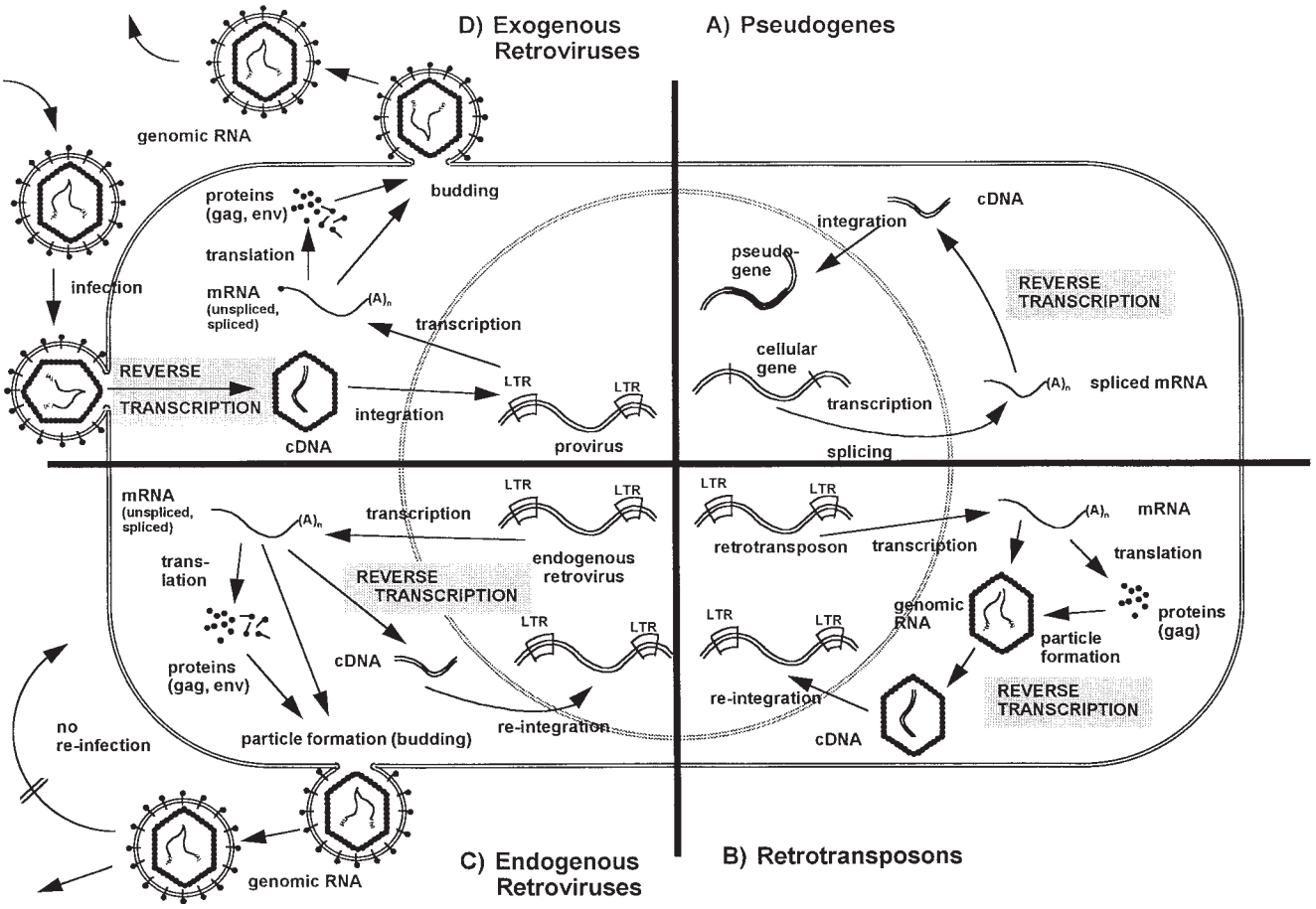

FIG. 2. Life cycles of retroelements. (*A*) Generation of pseudogenes. (*B*) Transposition of retrotransposons. (*C*) Expression and amplification of ERVs. (*D*) Replication cycle of exogenous retroviruses.

hybridization studies (24) revealed nine different families related to MMTV and to each other, including HTDV/HERV-K.

Taxonomy is an unresolved problem in HERV research. Exogenous retrovirus strains are generally designated according to their host and the disease they induce or are named after their discoverers. These criteria are difficult to apply to HERVs. Usually, they are already present in Old World monkeys, an association with a disease remains to be shown, and different proviruses which belong to the same family have been detected by independent investigators. Instead, many HERV proviruses were given arbitrary laboratory names. A tentative systematic nomenclature is based on the tRNA specificity of the primer binding site, using the one-letter code for the specific amino acid as a suffix to the acronym HERV (25). Limitations of this approach arise when several distantly related families have a very similar primer binding site or when cloned proviruses are either devoid of a 5′ end or have been only partially sequenced. Furthermore, frequent point muta-

Table 2. Characteristics of HERV families

Family	Prototype proviral clone (ref.)	Copy number	Genomic structure	mRNA expression	Protein expression
HERV-E	4–1 (17)	30–50	Defective provirus	Placenta, colon, breast cancer, brain, tumor cell lines	Not detected
HERV-R	ERV-3 (13)	1	Defective provirus, with *env*	High expression in placenta, low in normal tissues, fetal tissue, macrophage cell line U937	Env
HERV-I	RTVL-Ia (18)	3–25	Defective provirus	Not tested	Not detected
HERV-H	RTLV-H2 (19)	10^3–10^4	Transposon structure	Teratocarcinoma cell lines, placenta, tumor cell lines	Not detected
HERV-H	RGH2 (12)	50–100	Defective provirus	Teratocarcinoma cell lines	Not detected
ERV-9	γ Fix 1.1(14)	30–50	Defective provirus	Teratocaarcinoma cell lines, placenta	Not detected
HERV-P	HuERS-P1–1 (20)	10–20	Provirus (only LTR sequenced)	Not tested	Not tested
HTDV/HERV-K	HERV-K10 (21)	30–50	Defective (?) provirus, with all viral genes	High in teratocarcinoma cell lines, testicular tumors, low in placenta and normal tissue	Gag, cORF, protease, polymerase, Env
HERV-K(C4)	C4 (22)	30–50	Defective provirus	Not tested	Not tested

tions due to the lack of selective pressure on defective genomes are blurring the exact sequence of the primer binding site. All class II elements identified so far have a lysine primer binding site, reflecting their derivation from B- and D-type viruses.

HTDV/HERV-K, a Family Coding for Viral Particles

The first indication that retroviruses had not spared the human species came from electron microscopic surveys of human placentas. Retrovirus-like particles were observed budding at the basal membrane of syncytiotrophoblasts (reviewed in ref. 23). In addition, retrovirus-like particles were frequently detected in more than 20 testicular tumor cell lines derived from embryonic carcinomas or teratocarcinomas (26, 27). The latter are germ-cell tumors that have retained the potential to differentiate, for instance into trophoblastic cell lineages. Our group has designated these particles HTDV for *h*uman *t*eratocarcinoma-*d*erived *v*irus particles (27). Morphologically, the majority of these particles closely resemble those seen in the placenta: they lack an electron-lucent space between viral core and envelope and often seem to be arrested in the budding stages (ref. 28; see Fig. 3*a*). Rarely, mature forms with collapsed cores can be detected in ultrathin sections of teratocarcinoma cell lines. These morphological peculiarities may explain their hitherto apparent lack of infectivity, as transfer of HTDV to other cell lines has not yet been achieved (27).

Prior to the advent of PCR techniques it was practically impossible to identify which of the many HERV sequence families in the human genome codes for the HTDV particles. The RU5-PCR technique designed by our group to amplify retroviral transcripts by using primers with homology to viral primer binding sites (29) eventually allowed us to show that the endogenous retrovirus family HTDV/HERV-K is highly expressed in teratocarcinoma cell lines and that it codes for HTDV (30, 31).

The HTDV/HERV-K family occurs as about 25–50 full-length copies (15). In addition, about 10,000 solitary LTRs are scattered throughout the human genome (16). HTDV/HERV-K related sequences can be traced back to the time of divergence of Old and New World monkeys (15, 32, 33). The genomic distribution of HTDV/HERV-K proviruses appears to be nonrandom (34, 35). Ono *et al.* (21) published the sequence of a full-length provirus designated HERV-K10, which, although defective in *gag* and *env*, still serves as useful standard for sequence comparison.

Complex HTDV/HERV-K Proviral Organization and Transcripts. Our analysis of cDNA clones isolated from HTDV/HERV-K-expressing teratocarcinoma cells revealed ORFs for all viral genes (30, 36). The sequence as well as the genomic organization of HTDV/HERV-K proviruses resembles most closely that of type B/D retroviruses. *gag*, protease, and *pol* genes are present in three different overlapping ORFs requiring two frameshift events for translation of the Gag-protease-Pol protein precursor. Pol and Env reading frames overlap partially (Fig. 4). The proviruses have a long highly positively charged Env signal peptide (36) similar to those present in MMTV, JSRV, and nonprimate lentiviridae (37).

HTDV/HERV-K RNA expression is easily detectable in teratocarcinoma cell lines (29, 30) and is reminiscent of the pattern seen in complexly regulated retroviruses: full-length transcripts are accompanied by subgenomic *env* transcripts as well as alternatively spliced small mRNA species (Fig. 4). A 1.8-kb doubly spliced transcript covers most of the type 2-specific Env signal peptide (see Fig. 4). This transcript encodes an ORF designated cORF for central ORF (30, 36).

In many normal tissues, including the placenta, HTDV/HERV-K mRNA expression can be detected by using very sensitive methods such as RT-PCR (38, 39). Expression has also been observed after glucocorticoid stimulation of T47D, a cell line derived from a human mammary carcinoma (40). Although in these studies only full-length mRNAs have been demonstrated, recent RT-PCR-based experiments revealed the presence, at a very low level, of spliced HTDV/HERV-K transcripts in a variety of normal and tumor tissues (ref. 39 and unpublished results). In addition, transcripts longer than the expected full-length size can be detected in Northern blots (30, 40). They may represent readthroughs into adjacent cellular genes or initiation of transcripts by upstream cellular promoters in the sense as well as in the antisense orientation.

Expression of HTDV/HERV-K Proteins. In sharp contrast to other human endogenous retrovirus families which, in general, are highly defective, HTDV/HERV-K proviruses possess long ORFs in their viral genes. *gag*, protease, *pol*, *env*, and cORF genes have been expressed in pro- and eukaryotic expression systems to facilitate the study of protein expression and function and the production of antisera in animals (30, 36, 41–43).

The *Gag* proteins are produced as 76-kDa precursor proteins and cleaved into major core, matrix, and nucleocapsid components (31, 43). In teratocarcinoma cell lines the precursor is myristoylated (unpublished data), a prerequisite for transport to the plasma membrane and for particle production (44). In cell lysates, the precursor is the dominant viral protein, whereas in viral pellets the major core protein is more prominent. However, HTDV particles still contain a substantial

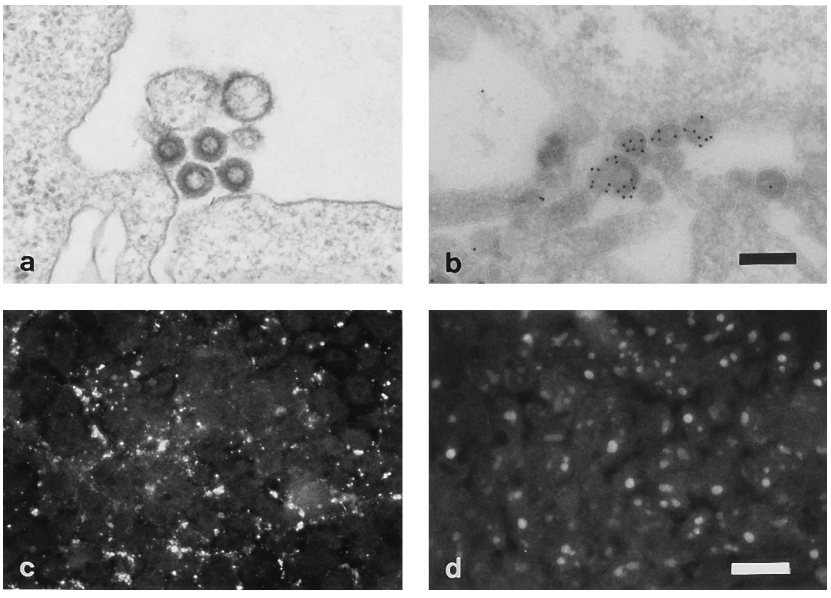

FIG. 3. HERV-K sequences code for HTDV. (*a*) HTDV particles produced by a teratocarcinoma cell line; ultrathin section of resin-embedded cells. (*b*) Ultrathin frozen section immunogold labeled with rabbit anti-HERV-K Gag antiserum. (*c*) Immunofluorescence with formalin-fixed GH teratocarcinoma cells and rabbit anti-HERV-K Gag antiserum. (*d*) Immunofluorescence with formalin-fixed GH cells and rabbit anti-HERV-K cORF antiserum. (*a* and *b*, bar represents 200 nm; *c* and *d*, bar represents 25 μm.) Micrographs courtesy of K. Boller, Paul-Ehrlich-Institut.

proportion of uncleaved or partially cleaved intermediates. Insufficient or aberrant cleavage may cause the morphological peculiarities (see Fig. 3*a*) and lack of infectivity of the particles. In this context it is interesting to note that simultaneous incorporation into HIV virions of intact and defective Gag proteins has been shown to lead to a dominant-negative phenotype lacking infectivity (ref. 45; unpublished data).

Gag proteins are easily detectable in teratocarcinoma cell lines by immunofluorescence as a particulate staining pattern at the cell membrane, compatible with an accumulation of Gag in viral particles (Fig. 3*c*). This interpretation has been supported by electron micrographs obtained after immunogold labeling of HTDV particles (refs. 30 and 31; Fig. 3*b*). In testicular tumors, Gag is also demonstrable in the cytoplasm by immunoperoxidase staining (43).

The presence of processed Gag proteins in teratocarcinoma cell lines indicates a functional HTDV/HERV-K *protease*. cDNA clones (unpublished data) as well as genomic proviruses amplified by PCR (46) could be shown to encode a functional enzyme.

The open reading frame of the *polymerase* gene codes for a 160-kDa protein. RT activity has been detected in virus particle preparations from the supernatant of teratocarcinoma cells (27). This activity was masked by the presence of a yet-unidentified cellular inhibitor specific for retroviral RTs (47). Although HTDV/HERV-K *pol* sequences were expressed as recombinant proteins in *Escherichia coli*, no RT activity could yet be detected (unpublished data). Endogenous RT-like enzymes may require special conditions which may be present only in the environment of the viral core.

In many tissues, the presence of *env* transcripts which remain undetectable in Northern blots can be shown by using the sensitive RT-PCR technique (39). In teratocarcinoma cells *env* transcripts comprise only a minor mRNA species due to the dominant splice donor in the signal peptide region. *env* is removed as an intronic sequence and an excess of the doubly spliced 1.8-kb cORF mRNA species is produced (36). This splicing pattern can be mimicked in eukaryotic expression systems. Using cDNA-derived *env* expression constructs under the control of the cytomegalo-virus (CMV) promoter, Tönjes and coworkers have demonstrated that in COS cells cORF is also the dominant splice product. However, cORF splicing in COS cells could be partially suppressed by inserting CMV intron A sequences 5′ of the *env* gene, eventually allowing the detection of Env protein production (ref. 41 and unpublished results). The Env precursor is slightly glycosylated, but it remains uncleaved and retained in the cytoplasm, a surprising observation because the consensus SU/TM cleavage site is present in the proviral sequence. At present, the reason for the failure to correctly process Env precursor protein remains unknown. In teratocarcinoma cells Env proteins are synthesized only at very low levels (unpublished data). Insufficient production of Env could be another reason for the lack of HTDV infectivity.

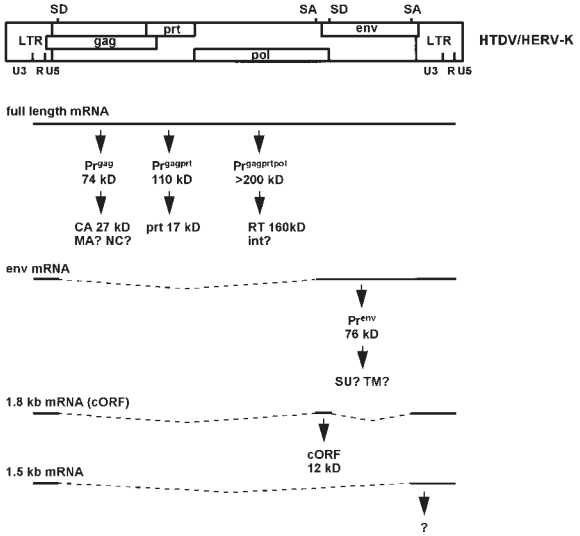

FIG. 4. HTDV/HERV-K: Genomic organization as well as the mRNA and protein expression pattern observed in teratocarcinoma cell lines. LTR, long terminal repeats consisting of stretches designated U3, R, and U5; gag, group-specific antigen gene; prt, protease gene; pol, polymerase gene; env, envelope gene; SD, splice donor; SA, splice acceptor; Pr, precursor protein; CA, major capsid protein; MA, matrix protein; NC, nucleocapsid protein; int, integrase; SU, surface protein; TM, transmembrane protein.

The doubly spliced cORF mRNA product is a 12-kDa two-exon protein: the first exon comprises two-thirds of the amino terminus of the Env signal peptide; the second exon is derived from a different reading frame in the 3′ part of *env* (36). In this respect, cORF resembles the ungulate lentivirus Rev proteins (48). Homology to Rev is also suggested by the presence of a nucleolar localization signal and, indeed, cORF accumulates in the nucleolus (see Fig. 3*d*). In addition, cORF is one of the most abundant gene products, as are the regulatory human lentivirus proteins Tat and Rev early after infection. Although cORF contains a domain with similarities to primate Rev effector domains, the spacing of leucine residues is slightly different (40) and it is therefore unlikely that the identified cORF protein can exert a Rev-like function (B. Cullen, personal communication).

Antibody Response to HTDV/HERV-K in Humans. Expression of HTDV/HERV-K proteins can also be detected by examining the humoral immune response. By using synthetic peptides, antibodies were detected at a very low frequency in normal blood donors consistent with the observation that in normal tissues HTDV/HERV-K is expressed at only a low level. In a survey of patient sera (Table 3), antibodies have been observed in leukemias, after pregnancies, and especially in patients suffering from testicular tumors (39, 43). Although antibody titers are elevated compared with normal blood donors, they hardly ever reach the titers observed after infection with exogenous retroviruses such as HIV. Nevertheless, it is intriguing that antibodies are made at all, since HERV proteins can also be regarded as self-antigens that should have induced tolerance. Interestingly, elevated antibody titers against Env proteins can be demonstrated (39, 49), although in the teratocarcinoma cell model Env cannot be detected on the cell surface. It will be important to learn whether the antibody response is directed against viral proteins that are expressed after induction of neonatal tolerance or whether they reflect mere crossreactivities with yet-unidentified cellular antigens. If it is not such a crossreactivity, the cell types producing Env need to be identified.

The appearance of Env in cell types other than teratocarcinoma cells indicates by a cell-type specific expression of a yet-undefined subset of HTDV/HERV-K proviruses that code for Env proteins which can be processed correctly, implying that different proviruses are regulated differently. To study this possibility, a number of LTRs were tested for promoter activity in reporter gene assays. In the particle-producing cell

Table 3. Immune response to HTDV/HERV-K

Source of serum tested	No. positive	No. tested	% positive
Testicular tumor patients	45	100	45
Lymphoma patients	31	120	26
Mammary carcinoma patients	0	11	0
HIV-1-positive patients	35	50	70
Pregnant women	3	8	38
Blood donors	1	30	3

Data courtesy of J. Denner, Paul-Ehrlich-Institut.

line GH, most of the LTRs tested could act as strong promoters, although completely inactive or weakly active LTRs were also detected. Inactive LTRs have accumulated specific point mutations compared with the active LTRs. In cell lines other than the teratocarcinoma lines, for instance in a hepatocarcinoma cell line, the activity of all LTRs tested was extremely low or undetectable, indicating that HTDV/HERV-K promoters are primarily upregulated in embryonic tissues (50). Further studies are needed to address the question whether certain proviruses are specifically activated in adult tissues and which cellular factors may influence this activity.

HTDV/HERV-K-Derived SINE-R Elements and Solitary LTRs, Footprints of Retrotransposition. In the human genome, another group of HTDV/HERV-K related sequences has been detected (6): the SINE-R elements. These elements are composed of a G+C-rich region (probably derived from a cellular gene) followed by a sequence from the 3′ end of the HTDV/HERV-K *env* region, part of the 3′ U3R, and a poly(A) tail. The putative origin of SINE-R elements may be a trans-splice event between a cellular and a viral transcript followed by retrotransposition and amplification to 5000 copies. Recently, some light was shed on the history of SINE-R evolution (51). These elements are of primate origin and first emerged after divergence of the orangutan lineage. Further retrotransposition events have occurred after the divergence of chimpanzees and human beings, as shown by the presence of a SINE-R element in the human but not chimpanzee C2 locus (51), which encodes a component of the complement cascade. The variable number of tandem SINE-R repeats within this locus contributes to the multiallelic restriction length polymorphism of C2 in the human population (51).

The presence in chimpanzees but not in humans of a solitary HTDV/HERV-K LTR in a triose-phosphate isomerase pseudogene (52) is apparently another footprint of a retrotransposition event during recent evolutionary history. Likewise, polymorphic variation in the human major histocompatibility complex locus *HLA-DQ* could be attributed to the presence or absence of two solitary HTDV/HERV-K LTRs (53), an indication that the human genome is still the target of changes by retrotransposition.

Biological Significance of Retroelements and Endogenous Retroviruses

Conferring Protection. HERVs may be regarded as sequences that were accidentally integrated into the genome of Old World progenitors of subhuman primates. They seem to be irrelevant to their hosts, as indicated by their rapid mutation and deletion. As HERVs are fossils and their exogenous counterparts probably have long vanished (or still remain to be detected), it is nearly impossible to trace back their putative former biological functions. Nevertheless, conclusions may be drawn from comparison with animal retrovirus model systems. Although the following ideas, per se, will be speculative, they might well explain the original function of endogenous retroviruses.

MMTV and JSRV are close relatives of HTDV/HERV-K. Both these virus strains exist as exogenous and endogenous proviruses in their hosts and induce cancers (mammary and pulmonary carcinomas, respectively). The viruses being devoid of oncogenes, their pathogenic potential presumably resides in the LTRs, which can enhance expression of adjacent cellular genes, for instance protooncogenes (enhancer activation, reviewed in ref. 54). Thus, tumor development depends on the proviral integration site.

Several subtypes of MMTV exist which differ especially in the LTR. Interestingly, the MMTV LTR sequence comprises an accessory gene with superantigen function, the *SAG* gene (55). *SAG* gene expression facilitates MMTV infection of T and B Lymphocytes and subsequently of the mammary gland. Endogenous MMTV expression leads to the elimination of the responsive T-cell repertoire during induction of self-tolerance. This confers protection against infection with exogenous counterparts but not with MMTV subtypes that differ in the *SAG* gene (56). Thus, in the MMTV system endogenization of MMTV subtypes seems to be not yet completed and resistance is only partially achieved. This hypothesis is supported by the observation that feral mice differ in MMTV copy number, integration sites, and subtypes of their endogenous proviruses (57).

BaEV, the baboon endogenous retrovirus (58), and RD 114, the homologous endogenous retrovirus of domestic cats and related species (59), are examples of an endogenization process that is probably completed. Although these ERVs are expressed in embryonic tissue such as placenta or can be induced in tissue culture, they no longer infect their native hosts, and no related exogenous strains have been identified. Thus, endogenous proviruses may protect their hosts against infection with a closely related exogenous retrovirus—for example, by receptor interference (reviewed in ref. 60) and superantigen-mediated depletion of susceptible host cells.

During evolution, resistance to superinfection by the pathogenic exogenous counterparts may have imparted a survival advantage to the progeny of those individuals in which integration into the germ cell lineage occurred. Such integration would have indirectly helped survival of retroviruses, which by virtue of their endogenous nature are no longer subject to the selective pressure previously exerted on their exogenous strains. Resistance to superinfection in the long term may contribute to the eradication of the exogenous counterparts.

In this respect three observations are noteworthy. (*i*) Although a variety of class I and class II HERVs exist, no human exogenous viruses have been detected that resemble these simply structured and probably more ancient oncoviridae. This may indicate that such viruses have been eliminated in human predecessor species. (*ii*) No major variations in copy number and integration sites of full-length HERVs have been observed in human DNA, indicating a stable balance, in contrast to the still evolutionarily developing MMTV system. (*iii*) No endogenous counterparts of exogenous lentiviridae and spumaviridae are known. Either these complexly regulated virus types cannot endogenize or these viruses are too young, in evolutionary terms, and endogenization (which is probably an extremely rare and slowly progressing process) has not yet occurred.

Many endogenous retroviruses belong to multicopy families, possibly reflecting (*i*) multiple successive infections of germ cells with different ancestors or with different subtypes of a retrovirus, (*ii*) intracellular retrotransposition, or (*iii*) passive amplification of elements in the context of spacious transposon units. These events are not mutually exclusive. Excessive genomic amplification of endogenous retroviruses could be hindered by a variety of mechanisms: (*i*) blocking new virus entry by receptor interference; (*ii*) rendering the newly acquired DNA innocuous by hypermethylation; (*iii*) excluding transcriptional activity from the reproductive tract by tissue-specific silencers; (*iv*) inactivation by deletions and mutations; or (*v*) excising virus information by homologous recombination between the identical proviral 5′ and 3′ ends, leading to the formation of solitary LTRs.

Shaping Genome Plasticity. Once HERVs have been integrated, they may have also contributed to the evolution of their hosts. Genomes are not static entities. In phylogeny, genomic changes are a precondition for selection and adaptation. While mutations are slow and therefore unsatisfactory tools for genomic modification, plasticity is more efficiently achieved by rearrangements driven by recombination and transposition. Reverse transcription may be instrumental in inducing variations, as approximately 10% of the human genome consists of reverse transcribed and transposed sequences (61). HERVs, together with retroposons and retrotransposons, may be the main source of RT activity.

Retroelements like solitary LTRs contribute to allelic variation in contemporary populations, as has been shown in the complement and *HLA* loci (51, 53). They can also serve as useful markers to study the evolution of those genes in the primate lineage (62, 63). Although it remains an enigma why

retroelements accumulate in hypervariable regions of the genome, it is suggestive that these elements actively contribute to variability by retrotransposition and recombination. Finally, retroelements, including HERVs, may turn out to be useful to construct genetic maps in the human genome project.

Retroelements themselves may undergo evolutionary changes. Adey *et al*. (64) reported that rodent LINE elements have successively acquired novel promoter sequences from non-LINE sources and that these altered structures have been subsequently amplified. Likewise, D. Mager and coworkers reported the existence of three subtypes of HERV-H LTRs which show minor variations in the sequence of U3, the main promoter region (reviewed in ref. 23). Proviruses with LTR subtypes I and II have been amplified to a high copy number early in the evolution of primates. Amplification of suppressive promotor sequences may be the cause of the diminished activity seen in these LTR subtypes. In contrast, proviruses with the third LTR subtype, Ia, which is not present in Old World monkeys, show significant promoter activity in a variety of cell lines. Acquisition of new sequences may be a consequence of nonhomologous recombination or trans-splice events prior to reverse transcription. Ongoing evolution of retroelements, especially of their regulatory sequences, suggests that they are neither static genes nor selfish DNA. Instead, they may serve some cellular function.

Significance of Retrotransposition. Retrotransposition is not an uncommon feature of cell physiology and can be monitored in experimental systems (65). In the *germ line*, such events will either be deleterious or remain fixed in the population. In this context it is worth reiterating that many human endogenous retrovirus families and LINEs are highly expressed in tissues or cell lines with embryonic characteristics. Recently, transpositions of LINEs were detected (*i*) as *de novo* insertions into the coding regions of factor VIII genes resulting in hemophilia A (66, 67): (*ii*) by disruption of the adenomatous polyposis coli gene in a colon cancer (68); (*iii*) by insertion into the *myc* locus in a breast cancer (69); and (*iv*) by insertion into exon 48 of the dystrophin gene (70). These germ-line transpositions were associated with a loss of normal gene function and have been identified because they resulted in disease.

Retrotransposition can also be associated with a gain of function—for example, specific gene expression, an intriguing example of which can be found in the amylase gene cluster. During the evolution of primates, insertion of a member of the endogenous retrovirus family HERV-E into the promoter region has probably provoked extensive rearrangement of this locus and an alteration of tissue-specific expression (71). Selective expression of a zinc finger protein in human hematopoietic cell lineages could be associated with the presence of an ERV 9 promoter (72), a phenomenon not observed in Old World monkeys. Although solitary LTRs generally have suffered extensive mutations and deletions, usually resulting in loss of promoter activity, they occasionally have retained or regained function as shown by the isolation of mRNA species initiated or polyadenylylated in inserted LTRs (reviewed in ref. 23).

Retrotransposition in *somatic cells* may have very different consequences for the cell and the individual. Whereas reintegration into introns would have no effect at all, reintegration into exons will usually interrupt ORFs, resulting in the loss of gene function and possibly cell death, which in itself is irrelevant for the individual. In contrast, destruction of tumor suppressor genes by insertional mutagenesis has been shown to contribute to the multistep process required for carcinogenesis. Insertion of retroelements into the promoter region of cellular genes may eventually lead to overexpression and may contribute to tumor development if proto-oncogenes are involved. Viral enhancer elements may also alter gene regulation. Examples of such events have been elucidated in animal tumors (reviewed in ref. 54).

Significance of Protein Expression. Cell physiology may be influenced not only by chance retrotransposition but also by the significant pathological potential that resides in retroviral Env proteins. It has been shown that purified transmembrane envelope proteins as well as peptides corresponding to a highly conserved transmembrane domain are immunosuppressive (73). Such immunosuppressive peptides of type C and type D retroviruses as well as of HIV inhibit T- and B-cell activation (74). In addition, retroviral superantigens such as the *SAG* gene product of MMTV (55) or the *gag* gene of the murine AIDS-associated virus (75) can lead to massive T-cell stimulation and apoptosis. Recently, the possible involvement of a superantigen in the onset of diabetes was discussed (76).

Hosts should be tolerant to ERV self-antigens. In sheep infected with exogenous JSRV, circulating antibodies reacting with viral antigens are not detectable, indicating tolerance due to the presence of the closely related endogenous counterparts. However, in human beings, an immune response to HTDV/HERV-K proteins was easily detectable in patients with certain tumors (refs. 39, 43, and 49; see Table 3).

As mentioned above, mRNA products longer than full-length HTDV/HERV-K transcripts are present. It will be of interest to elucidate whether such transcripts possess coding capacity. If viral–cellular fusion products are made, studies have to be initiated to investigate their pathophysiological effects.

The notion that HERV proteins may serve a specific gene function in their host is supported by the demonstration that ORFs are still intact and have retained coding capacity despite the extensive mutations and deletions normally associated with endogenization of retroviruses. As mentioned above, HERVs are preferentially expressed in embryonic tissues. An interesting example stems from the single-copy gene HERV-R. HERV-R Env protein is induced to high levels during differentiation of syncytiotrophoblasts (77, 78). The placenta is a tissue with pronounced fusogenic and immunosuppressive properties, and retroviral Env proteins are known to possess such domains. The mass production of HERV-R Env protein detected in the syncytiotrophoblast layer therefore suggests a possible involvement in normal placenta function (79).

Outlook

In this review we tried to summarize present knowledge about retroelements and their biological relevance. We have described in some detail the biologically most active endogenous retrovirus family HTDV/HERV-K which has retained long ORFs and the capacity to be expressed at the RNA and protein levels, inducing an immune response. A conceivable involvement of HERV families in pathophysiological processes, if any, remains to be demonstrated. The following investigations may be seminal for an improved understanding of the biological significance of expressed HERV sequences.

(*i*) The search for complete proviruses should be continued not only for HTDV/HERV-K but also for other HERVs, especially for those which are so far only partially characterized. Virus particles have been observed in many other cell lines (reviewed in ref. 3), and it is conceivable that HTDV/HERV-K does not code for all of them. The additional sequence information generated will also help to unravel phylogenetic relationships.

(*ii*) Studies should be continued to investigate expression of HERV genes at the RNA and protein level. Proteins should be checked for putative functions in cell physiology and in pathological conditions. RT-PCR techniques have facilitated expression studies, but the extreme sensitivity of this method precludes instant interpretation of its relevance. RNA expression might be too low for protein production, whereas demonstrable protein synthesis suggests possible biological significance.

(*iii*) Retrotransposition and its impact on the genome will remain a fascinating topic to investigate. Such events most probably will not be detected by virologists, but by geneticists or physicians studying the genetic origin of disease development in individual patients. Demonstration of a functional human RT gene will significantly strengthen the hypothesis that HERVs play a decisive role in retrotransposition.

(*iv*) Studies should be intensified to search for cellular genes that are influenced or controlled by HERV enhancers or promoters, by HERV untranslated regions (UTRs), or by polyadenylation signals. The demonstration of viral and cellular fusion proteins would come as no surprise, and their pathogenic potential, as deduced from studies of animal tumor viruses, would be considerable.

(*v*) As retroviruses have the ability to recombine with one another and with endogenous sequences, studies should be initiated to elucidate whether HERVs represent risk factors in gene therapy. On the other hand, the potential to recombine may be exploited for site-directed integration by including HERV sequences in retroviral vectors (80).

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