

Divergent Patterns of Recent Retroviral Integrations in the Human and Chimpanzee Genomes: Probable Transmissions between Other Primates and Chimpanzees†

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The human genome is littered by endogenous retrovirus sequences (HERVs), which constitute up to 8% of the total genomic sequence. The sequencing of the human (*Homo sapiens*) and chimpanzee (*Pan troglodytes*) genomes has facilitated the evolutionary study of ERVs and related sequences. We screened both the human genome (version hg16) and the chimpanzee genome (version PanTro1) for ERVs and conducted a phylogenetic analysis of recent integrations. We found a number of recent integrations within both genomes. They segregated into four groups. Two larger gammaretrovirus-like groups (PtG1 and PtG2) occurred in chimpanzees but not in humans. The PtG sequences were most similar to two baboon ERVs and a macaque sequence but neither to other chimpanzee ERVs nor to any human gammaretrovirus-like ERVs. The pattern was consistent with cross-species transfer via predation. This appears to be an example of horizontal transfer of retroviruses with occasional fixation in the germ line.

Human endogenous retrovirus sequences (HERVs), which have become trapped as Mendelian genes and fixed in the germ line (9), constitute 7 to 8% of the human genome (8, 26). The sequencing of the human (*Homo sapiens*) genome (26) and the draft sequence of the chimpanzee (*Pan troglodytes*) genome (16) have facilitated the evolutionary studies of ERVs and related sequences. The *Homo-Pan* speciation has been estimated to have occurred between 4.6 and 6.2 million years ago (15). The genomic nonidentity is approximately 1.2% (15, 17) up to 5% (14), depending on analysis methods. We used a newly developed bioinformatic tool, RetroTector (G. O. Sperber, T. Airola, P. Jern, A. Castell, and J. Blomberg, unpublished data), to screen both the human genome (version hg16) and the chimpanzee genome (version PanTro1) for ERVs. We have successfully used RetroTector-derived data in several papers; the program validation can be traced in these papers and their respective supplemental materials (1, 27–29, 42). The differences in ERV content serve as indicators of dynamic genomes. The proviral long terminal repeats (LTRs) are identical at the integration moment (50). We analyzed recent provirus integrations with less than 2% LTR difference. With a typical value of 0.2% substitutions per million years (34), this LTR difference served as an approximation to 5 million years and the *Homo-Pan* sp. split. In this paper, we refer to these proviruses as “recent,” knowing that postintegrational mutations can accumulate at different rates depending on the genetic environment (25, 33, 60). The potential effects of retroviral integrations have been studied in several papers. The effects include addition of promoters and enhancers (43) and

introduction of alternative splice patterns (18). Recently, it was also shown that the human L1 retrotransposon generates a somatic variation which influences both gene expression and cell differentiation (41). Generation of somatic variation may also apply to retroviruses (57).

In this paper, we demonstrate unique sequences in the human and chimpanzee genomes. We found a difference in recent activity between the beta-like and gamma-like retroviruses. This difference applied to both genomes inversely, with one group expanding in each genome. It indicates the importance of environmental factors and random reactivation events in preexisting elements for determining the retroviral genetic setup. Several cross-species transfers of nonhuman, nonchimpanzee primate gamma-like retroviruses to chimpanzee have occurred since the *Homo-Pan* sp. split.

MATERIALS AND METHODS

Bioinformatics. Alignments were performed using ClustalX 1.83 (51) with default settings. The neighbor joining (NJ) dendrogram (Fig. 1) was constructed in the MEGA software package (32) using pairwise deletions, p-distance, and 500 bootstraps. The bootstrap values are, due to limited space in Fig. 1, presented in an NJ cladogram rooted on midpoint in Fig. S1 in the supplemental material. Sequences retrieved by RetroTector (Sperber et al., unpublished data) were verified by BLAT search (<http://genome.ucsc.edu/cgi-bin/hgBlat>) against the respective genomes. tBLASTn searches (<http://www.ncbi.nlm.nih.gov/BLAST/>) using putative Pol (“puteins”) were conducted with the following search criteria for PanTro1: Primates NOT Pan Troglodytes [ORGN] alt. for hg16: Primates NOT Homo Sapiens [ORGN]. The rooted NJ cladogram (see Fig. S2 in the supplemental material) was constructed using pairwise deletions, p-distance, and 1,000 bootstraps. The similarity matrix (Fig. S2 in the supplemental material) was calculated from the cladogram data using pairwise deletions and PAM250 scoring. The similarity score of the shortest sequence versus itself was set to 100%. Sequences included into respective groups were at least 80% similar and applied to the clades in Fig. 1. The gammaretroviral groups were confirmed against a maximum parsimony tree constructed in PAUP4.0 (see Fig. S3 in the supplemental material).

The program RetroTector was used to screen both the human (hg16) and chimpanzee (PanTro1) genomes. Briefly, RetroTector recognizes consensus motifs and constructs putative ERV proteins (“puteins”) from the different reading

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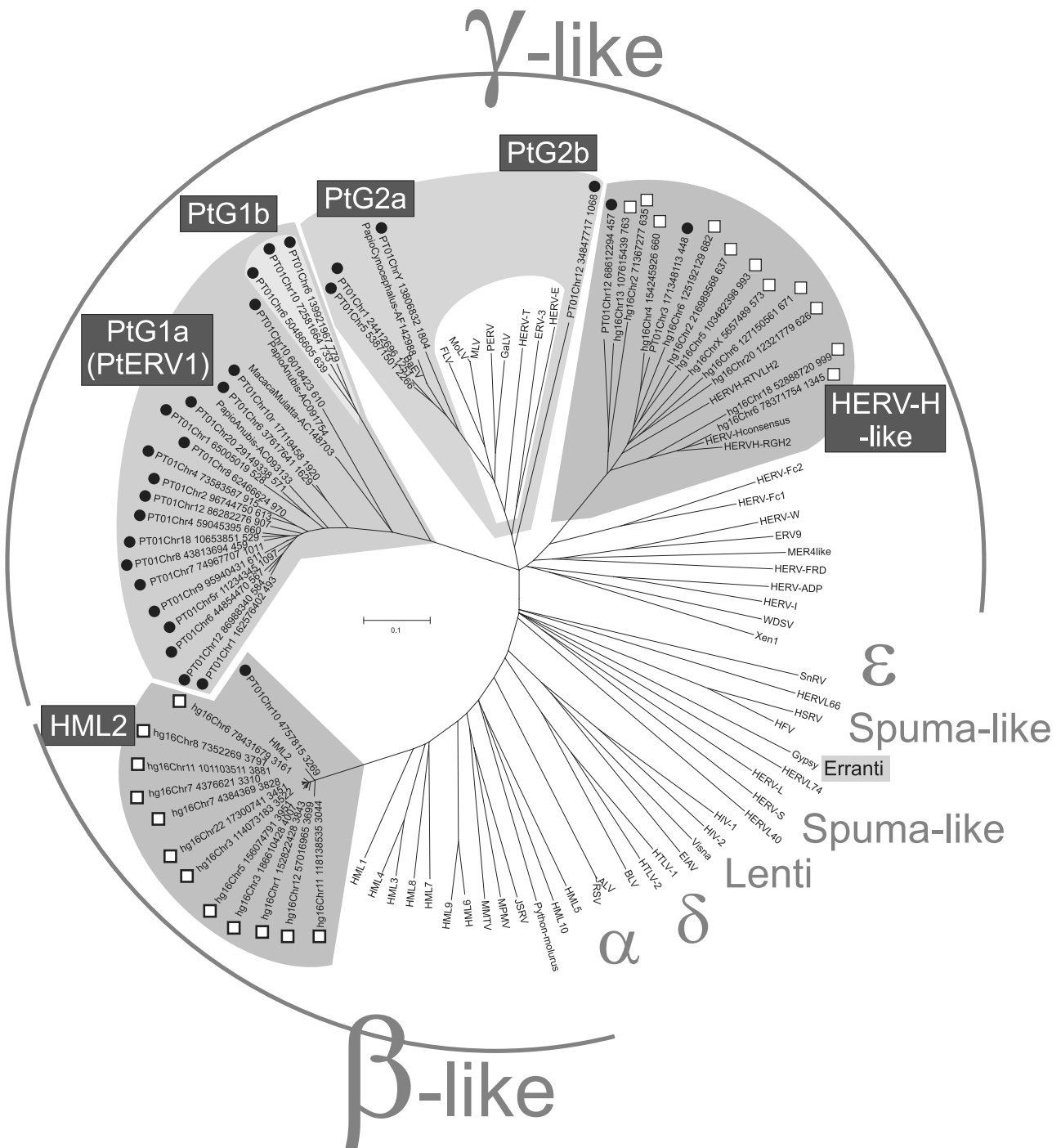


FIG. 1. Pol NJ phylogeny of recently integrated hg16 proviruses (indicated by white boxes), PanTro1 proviruses (indicated by black circles), and reference retroviral sequences using the entire proteins or “puteins.” Shadows indicate the different groups discussed in the text. Abbreviations are spelled out in the last paragraph of Materials and Methods. Bootstrap supports for the corresponding NJ cladogram rooted on midpoint are presented in Fig. S1 in the supplemental material.

frames in the gene candidates. The program uses codon statistics, frequency of stop codons, and alignment to known retrovirus proteins to approximate an original open reading frame (ORF). The Pol includes a number of conserved motifs in both reverse transcriptase (RT) and integrase (IN) that facilitates the RetroTector computations. The RetroTector RT1-5 motifs correspond to the conserved Pol RT motif numbers 1 and 3 to 6, presented by Xiong and Eickbush (59). This RetroTector multifactorial sequence analysis is more robust to poor sequence quality than traditional BLAST alignments (Sperber et al., unpublished

data), which are based on similarities to predefined query sequences. Validation of the RetroTector-derived data can be found in the supplemental data of several papers (1, 27–29, 42).

Sequences and accession numbers. The genome sequences for human (hg16) and chimpanzee (PanTro1) were retrieved from the UCSC Genome Browser (<http://genome.ucsc.edu/>).

GenBank accession numbers or chromosomal positions in hg16 for reference (putein) sequences used in the analysis were as follows: avian leukosis virus

TABLE 1. Properties of recent ERV integrations in the human (hg16) and chimpanzee (PanTro1) genomes

Element detected	No. in hg16 (24) ^a		No. in PanTro1 (36) ^a	
	Beta (B)	Gamma (C)	Beta (B)	Gamma (C)
ERVs detected	12	12	1	35
Detected <i>gag</i>	12	10	1	18
Detected <i>pro</i>	12	4	1	9
Detected <i>pol</i>	12	11	1	27
Detected <i>env</i>	12	2	1	22
Detected "LTR- <i>gag</i> - <i>pro-pol-env</i> -LTR"	12	1	1	1

^a Numbers in parentheses indicate the number of ERVs with <2% LTR nonidentity. "Beta" and "Gamma" indicate beta- and gamma-like genera assigned by RetroTector.

(ALV) (NC001408), Rous sarcoma virus (RSV) (NC001407), mouse mammary tumor virus (MMTV)/(MPMV) (NC001503), Mason-Pfizer monkey virus (MMTV)/(MPMV) (NC001550), Jaagsiekte sheep retrovirus (JSRV) (M80216), HML1 (chr19-21849393), HML2 (chr11-101600013), HML3 (chr1-48344461), HML4 (chr8-75679221), HML5 (AC004536), HML6 (consensus), HML7 (chr6-121300220), HML8 (chr3-131452286), HML9 (chr9-62700428), HML10 (chr6-32017925), HERV-H (consensus), HERV-H/RGH2 (D11078), HERV-H/RTVLH2 (M18048), HERV-Fc1 (AL354685), HERV-Fc2 (AC019088), HERV-W (chr7-9105739), ERV9 (AC073410), ERV3 (Chr7-63865366), HERV-E (M10976), murine leukemia virus (MLV) (NC001501), Moloney murine leukemia virus (MoMLV) (AF033811), baboon endogenous retrovirus (BaEV) (D10032), gibbon ape leukemia virus (GaLV) (M26927), HERV-ADP (AC005741), HERV-FRD (AC004022), HERV-I (chr16-72821350), HERV-T (chr14-104635791), HERV-S (AC004385), feline leukemia virus (FLV) (NC001940), porcine endogenous retrovirus (PERV) (AJ293656), walleye dermal sarcoma virus (WDSV) (NC001867), *Xenopus laevis* endogenous retrovirus (Xen1) (AJ506107), snakehead fish retrovirus (SnRV) (NC001724), bovine leukemia virus (BLV) (NC001414), human T-cell leukemia virus 1 (HTLV-1) (NC001436), HTLV-2 (NC001488), Gypsy (AJ000387), HERV-L (RepBase), human spumaretrovirus (HSRV) (AF033816), human foamy virus (HFV) (NC001736), MER4like (chr13-54208300), HERVL66 (RepBase), HERVL74 (RepBase), HERVL40 (RepBase), and *Python molurus* endogenous retrovirus (AAN77283).

RESULTS AND DISCUSSION

Genomic differences in recent ERV integrations. In a screening for ERVs in the human (*Homo*) genome (version hg16) and the chimpanzee (*Pan*) genome (version PanTro1), using the RetroTector bioinformatic tool, we found 24 integrations in *Homo* and 36 integrations in *Pan* with less than 2% LTR divergence (Table 1). As mentioned, this LTR difference corresponds to 4 to 5 million years of selection-neutral evolution, approximating the *Homo-Pan* sp. split, based on a typical value of 0.2% substitutions per million years (34).

The 51 *pol*-containing proviruses with <2% LTR divergence were analyzed further. The whole RetroTector-derived hg16 and PanTro1 ERV nucleotide sequences (Table 2) were used in BLAT searches against both the *Homo* and *Pan* sp. genomes to verify the positions and uniqueness (i.e., occurring in only one or the other species [underlined in Table 2]). Before a *Homo* sp. ERV was labeled as unique, we analyzed the occurrence and matching of orthologous flanking sequences of the proviruses in the *Pan* sp. Proviral integrations with doubtful chromosomal flanking sequences in the opposite species were not considered unique. False conclusions due to poorly sequenced or missing chromosomal regions were thus minimized. Using these rather stringent criteria, 31 of the 51 *pol*-containing proviruses were assessed to be unique to the

respective genomes and to have integrated after the *Homo-Pan* speciation (underlined sequences in Table 2). These are in this paper operationally referred to as "recent" integrations. Further, the uniqueness of 10 integrations was uncertain (Table 2). Their BLAT results are presented in Fig. S6 in the supplemental material. In an attempt to test the <2% LTR divergence limit, an extended analysis of the human genome (hg16), using <3% LTR divergence, resulted in 4 additional beta-like and 19 additional gamma-like Pol-containing proviral integrations. Among those integrations, only two additional uncertain unique gamma-like proviruses (HERV-H like) at chromosomes 3p12 and 6p22 were detected (data not shown), and two probably unique beta-like (HML2) proviruses were found at chromosomes 19p13 and 3q21 (data not shown). This indicates that the LTR divergences are not exact measures of integration times. They are probably influenced by recombination events or gene conversions (24, 30) but useful in screenings. The extended analysis, in this case by an additional 1% LTR divergence, thus resulted in an increasing proportion of uncertain proviruses (Fig. S6 in the supplemental material) and very little unique integration.

Among the recent integrations, with <2% LTR divergence, we found mainly beta-like proviruses in hg16, whereas the recent PanTro1 integrations were dominated by gammaretrovirus-like integrations (Fig. 1). Given that the International Committee on Taxonomy of Viruses (ICTV) does not classify endogenous retroviruses, the beta-like and gamma-like proviral integrations described here will, with support from their positions in the phylogenetic analyses (Fig. 1 and Fig. S1 to S3 in the supplemental material) and their genomic structures (see Fig. S4 in the supplemental material), henceforth in this paper be referred to as betaretroviruses and gammaretroviruses, respectively. Only a few of the recently integrated gammaretroviral sequences in hg16 had full retroviral gene structures (Tables 1 and 2). In contrast, the recent betaretroviruses in hg16 (Tables 1 and 2) were all full-length, with one or several ORF genes per provirus. We used the entire reconstructed Pol protein (potein) sequences as computed by RetroTector (23 for hg16 and 28 for PanTro1) together with reference sequences and constructed an unrooted NJ dendrogram (Fig. 1). Bootstrap supports are presented in a corresponding NJ cladogram (Fig. S1 in the supplemental material). The gammaretroviral NJ clades were similar to those of a maximum-parsimony analysis (Fig. S3 in the supplemental material).

In the *Homo* sp., one betaretroviral and one gammaretroviral group were detected (Fig. 1). The groups were selected using an 80% Pol similarity criterion. All recent human betaretroviruses were members of the HERV-K(HML2) group (2), hereafter called "HML2." They were 97.5 to 99.9% similar to each other in Pol (see the similarity matrix in Fig. S2 in the supplemental material) and 98.7 to 99.8% similar to an HML2 consensus sequence (V. Blikstad, G. O. Sperber, and J. Blomberg, unpublished). This homogeneous subgroup of HML2 corresponds to what was earlier named "human-specific HERV-K" (4, 38, 39). All recent human gammaretroviruses grouped within the HERV-H-like group (28).

In the *Pan* sp., the recent integrations were dominated by 27 gammaretroviral sequences, but there was also one betaretroviral sequence (Fig. 1). The single recent betaretroviral *Pan* sp.

TABLE 2. Grouping and characterization of recent Pol-containing ERV integrations in the *Homo* (hg16) and *Pan* (PanTro1) genomes

Sequence from Pol protein tree (Score > 300, LTR nonidentity < 2%) (species "unique") ^a	Genomic positions (PanTro1/hg16) ^b	Nucleotide frequency (%)				Chain (genus)	PBS	Stops and frameshifts (genus)				LTR nonidentity (%)	BLAT 5' and 3' LTRs >98% of max. score	Best Pol hits in tBLASTn (against nr database)	
		A	T	G	C			gag	pro	pol	env				
PIG1a															
pt01Chr1_162576402_493	chr1: 162576402(-)	26.5	21.7	23.7	28.2	gamma (C)		12 (C)	6 (C)		1.58	4	Using 75% Pol consensus		
pt01Chr1_65005019_528	chr1: 65005019(-?)	26.1	22	23.4	28.5	gamma (C)		9 (C)	4 (C)		0.48	4	ACI48703 <i>Macaca mulatta</i>		
pt01Chr10_6018423_610	chr10: 6018423(-)	27.3	22.5	22.4	27.8	gamma (C)	P	11 (C)	4 (C)		0.53	9	ACO93133 <i>Papio anubis</i>		
pt01Chr10_1711172_1920	chr10: 1711172(-)	26.9	21.5	23.6	28	gamma (C)	P	2 (C)	0 (C)	7 (C)	1.99	2	AFI42988 <i>Papio cynocephalus</i>		
pt01Chr12_86282276_907	chr12: 86282276(-)	26.2	21.5	24	28.3	gamma (C)	P	4 (C)	2 (C)	8 (C)	0.98	19			
pt01Chr12_86981358_584	chr12: 86981358(-)	26.0	21.8	23.8	28.4	gamma (C)	P	10 (C)	1 (C)	10 (C)	0.27	19			
pt01Chr18_10653851_529	chr18: 10646172(-)	25.6	21.6	24.5	28.3	gamma (C)		8 (C)	4 (C)	8 (C)	0.97	20			
pt01Chr2_96744750_613	chr2: 96737061(-)	24.3	22.1	25.1	28.4	gamma (C)		7 (C)	3 (C)	7 (C)	0.5	2			
pt01Chr20_29149338_571	chr20: 29142410(-)	27.1	21.6	23.3	28	gamma (C)		11 (C)	4 (C)	11 (C)	1.22	4			
pt01Chr4_59045395_660	chr4: 59038147(-)	26.1	21.6	24.1	28.1	gamma (C)		5 (C)	4 (C)	5 (C)	1.23	8			
pt01Chr4_73583587_915	chr4: 73584507(-)	26.7	21.4	23.6	28.3	gamma (C)		6 (C)	8 (C)	6 (C)	0.52	13			
pt01Chr5r_11234345_1097	chr5r: 11234345(-)	25.9	21.5	24.2	28.4	gamma (C)	P	3 (C)	2 (C)	3 (C)	0.76	17			
pt01Chr6_37617641_1629	chr6: 37617641(-)	26.7	21.5	23.5	28.3	gamma (C)		4 (C)	0 (C)	4 (C)	0.79	4			
pt01Chr6_44854470_567	chr6: 44854470(-)	26.8	21.7	23.2	28.3	gamma (C)		4 (C)	7 (C)	7 (C)	1.06	2			
pt01Chr7_74967707_1011	chr7: 74960368(-)	27.2	21.4	23.4	28.1	gamma (C)	P	2 (C)	2 (C)	4 (C)	0.79	8			
pt01Chr8_43813694_459	chr8: 43806427(-)	27.7	21.4	23	27.9	gamma (C)		11 (C)	5 (C)	11 (C)	0.95	3			
pt01Chr8_62466624_970	chr8: 62466624(-)	25.2	21.2	24.9	28.7	gamma (C)	P	5 (C)	1 (C)	1 (C)	0.73	25			
pt01Chr9_95940431_611	chr9: 95940431(-)	26.2	21.6	24.1	28.2	gamma (C)		7 (C)	9 (C)	7 (C)	0.98	8			
PIG1b															
pt01Chr10_72581664_733	chr10: 72573964(-)	25.6	22	24.2	28.2	gamma (C)		4 (C)	8 (C)	7 (C)	0	4	Using pt01Chr6_139921967_779		
pt01Chr6_139921967_779	chr6: 139915850(-)	24.9	22.5	24.6	28	gamma (C)		2 (C)	2 (C)	7 (C)	0.74	11	ACI48703 <i>Macaca mulatta</i>		
pt01Chr6_50486605_639	chr6: 50486605(-)	25.7	22.6	23.9	27.8	gamma (C)		7 (C)	3 (C)	7 (C)	0.49	5	ACO91754 <i>Papio anubis</i>		
PIG2a															
pt01ChrY_13806832_1804	chrY: 13806832(-)	25.7	21.1	23.9	29.3	gamma (C)	P	10 (C)	0 (C)	3 (C)	0	2	Using pt01Chr5_53871501_226		
pt01Chr1_24412696_1251	chr1: 24412696(-)	26.3	20.9	24.1	28.7	gamma (C)	P	3 (C)	1 (C)	7 (C)	0.29	4	AFI42988 <i>Papio cynocephalus</i>		
pt01Chr5_53871501_2266	chr5: 53871501(-)	25.7	22.1	23.6	28.6	gamma (C)	P	6 (C)	0 (C)	9 (C)	1.67	4	ACI48703 <i>Macaca mulatta</i>		
PIG2b															
pt01Chr12_34847717_1068	chr12: 34840755(-)	25.9	22	23.6	28.5	gamma (C)	P	1 (C)	1 (C)	8 (C)	0.79	4	Using pt01Chr12_34847717_1068		
HML2															
pt01Chr10_4757815_3269	chr10: 4757815(-)	32.2	26.1	21.3	20.4	beta (B)	K	6 (B)	3 (B)	6 (B)	0.63	4	ACI48703 <i>Macaca mulatta</i>		
hg16Chr1_152822428_3883	(-)/1q22	32.2	26.2	21.3	20.3	beta (B)	K	3 (B)	0 (B)	1 (B)	0.21	28	ACO91754 <i>Papio anubis</i>		
hg16Chr11_101103511_3881	(-)/11q22	32.2	26	21.3	20.5	beta (B)	K	3 (B)	0 (B)	1 (B)	0.41	61	Using pt01Chr10_4757815_326		
hg16Chr11_118138535_3044	(-)/11q22	32.1	26.2	21.4	20.3	beta (B)	K	3 (B)	0 (B)	7 (B)	0.41	4	ACO16577 <i>Homo sapiens</i>		
hg16Chr12_57016965_3699	(-)/12q14	32.3	26	21.2	20.5	beta (B)	K	2 (B)	0 (B)	4 (B)	0.41	58			
hg16Chr22_17300741_3451	(-)/22q11	32.2	26.1	21.3	20.4	beta (B)	K	2 (B)	1 (B)	3 (B)	0.41	21			
hg16Chr3_114073183_3522	(-)/3q13	32.3	26.1	21.3	20.4	beta (B)	K	2 (B)	2 (B)	2 (B)	0	106			
hg16Chr3_186610428_4007	(-)/3q27	32.2	26.2	21.1	20.4	beta (B)	K	2 (B)	1 (B)	1 (B)	0.31	11			
hg16Chr5_156074791_3951	(-)/5q33	32.2	26.2	21.3	20.3	beta (B)	K	2 (B)	0 (B)	1 (B)	0.21	24			
hg16Chr6_78431679_3161	(-)/6q14	32.2	25.9	21.4	20.5	beta (B)	K	2 (B)	0 (B)	3 (B)	0.31	33			
hg16Chr7_4376621_3310	(-)/7q22	32.1	26.1	21.4	20.5	beta (B)	K	3 (B)	0 (B)	1 (B)	0.86	4			
hg16Chr7_4384369_3828	(-)/7q22	32.4	25.8	21.3	20.5	beta (B)	K	3 (B)	1 (B)	1 (B)	0.21	4			
hg16Chr8_7352269_3797	(-)/8p23	32.3	25.9	21.3	20.5	beta (B)	K	2 (B)	1 (B)	1 (B)	1.36	16			

retroviruses (including MLV and BaEV), despite sequence similarity to another baboon ERV. There are thus distinct gammaretrovirus-like baboon ERVs as well (see below).

Horizontal transfers. There were signs of cross-species transfer involving the PtG retroviruses. We performed tBLASTn searches against the whole nonredundant database at GenBank with a consensus of the aligned PtG1a Pol sequences and with original Pol sequences to represent PtG1b, PtG2a, and PtG2b (Table 2). A few primate nonchimpanzee sequences, yielding high tBLASTn scores (data not shown), proved to be closely similar to the Pol of each PtG group (Table 2). They grouped as novel gammaretroviruses in Fig. 1. Also, they had typical gammaretroviral gene structures, with *gag*, *pro*, and *pol* in one reading frame separated from *env* and no obvious accessory genes (see Fig. S4 in the supplemental material). It was further noted that the human retroviral sequences had primer binding sites (PBSs) complementary to either Lys-tRNA in HML2 or His-tRNA in the HERV-H-like group, while detected PBSs in the recent *Pan* sp. ERV groups (PtG1 and PtG2) were complementary to Pro-tRNA (Table 2). Other primate gammaretroviral sequences, like HUERSP3 and a number of ERV3-like sequences (1), also have a Pro-PBS but were only 50 to 76% similar in Pol to PtG1 (data not shown).

The nonchimpanzee sequences were also included in the Pol similarity matrix (Fig. S2 in the supplemental material). Both PtG groups were >80% similar in Pol to both murine and feline leukemia viruses (Fig. 1). The PtG1 elements were highly (84 to 96%) similar in Pol to two previously not described ERVs: a baboon (*Papio anubis*) sequence, clone AC093133, and a macaque (*Macaca mulatta*) sequence, clone AC148703 (Fig. S2 in the supplemental material and Table 2). The PtG2 elements were similar to the baboon endogenous retrovirus (BaEV) and to a *Papio cynocephalus* sequence, clone AF142988 (Fig. S2 in the supplemental material). The three PtG2a elements were approximately 96% similar to each other (Fig. S2 in the supplemental material). They were more than 92% similar to BaEV Pol (56) and to the *Papio cynocephalus* ERV Pol (Fig. 1 and Fig. S2 in the supplemental material). Further, they were 85% similar to MLV Pol. The single PtG2b sequence was more separate in the retroviral tree (Fig. 1) but was 93% similar to a *Papio anubis* ERV in clone AC091754 and 88% similar to MLV. A supplementary comparison of the PtG groups with a range of previously described BaEV-related Pol (RT, 108 amino acids) sequences (55), obtained through the courtesy of Antoinette van der Kuyl, showed that the PtG1 group also was similar to *Papio* and *Colobus* ERVs other than those described in Table 2 (see Fig. S5 in the supplemental material). The PtG2a elements grouped within the BaEV (55) clade. The PtG2b element grouped together with the *Papio anubis* sequence, however inconsistently (see above) (Fig. S1 to S3 in the supplemental material). Although a recent integration, it came out in an ancestral position relative to much older gamma-like HERVs, relatively close to HERV-E (Fig. 1 and Fig. S1 to S3 in the supplemental material). This could mean that more-or-less close relatives of gammaretrovirus-like HERVs may still be spreading among primates.

Subsequent LTR analysis (Fig. S5 in the supplemental material) showed that the PtG1 elements were highly similar (average, 88% nucleotide identities using pairwise deletion) to the chimpanzee LTR homologues of the colobus CPC-1 pro-

viruses described by Bonner et al. (10). PtG1 LTRs were also similar to the macaque MAC-1 LTR (average, 80%) but less similar to the colobus CPC-1 LTR (average, 58%). However, the reference LTR sequences were not full-length and therefore merely indicate kinship between the ERVs. Due to separate ERV data sets and genes, a strict comparison between the Pol and LTR phylogenies (Fig. S5 in the supplemental material) is not possible. What is clear is that there are several novel sequences in the chimpanzee. They were probably transmitted to chimpanzees several times, in a complex way, from other primates in the recent past. We here demonstrate close similarities of the PtG groups to at least three different primate ERV groups, including BaEV.

The PtG1a, the PtERV1 (61), and the CPC-1 elements may derive from the same virus. CPC-1 was earlier described to occur in chimpanzee and gorilla but not in human, gibbon, and orangutan. Further, it was supposed to have transmitted to chimpanzees from colobus (10). Although mostly based on hybridization data derived from the presequencing era, that information on type C retroviral sequences in chimpanzees (6, 12) supports our findings. It was here corroborated and extended by a bioinformatic approach. The transmission hypothesis gained support from the (however limited) Pol and LTR phylogenies (Fig. S5 in the supplemental material), in addition to the pairwise LTR similarities from that alignment (see above).

Based on *gag* and *env* analyses, Yohn et al. recently showed that the PtERV1 elements (similar to our PtG1a group in the Pol analysis) (Fig. S2 in the supplemental material) may possibly have more than one origin (61). Exogenous retroviruses from at least three host groups, including (i) chimpanzee together with gorilla, (ii) baboon, and (iii) macaque, were suggested to have contributed. Their phylogenetic trees differed from generally accepted primate species trees, thus indicating horizontal transfer (61). Our data agree with those results. Although theoretically transmissions could first have reached chimpanzees and then gone from chimpanzees to other primates, the simplest explanation is transmission of nonhuman, nonchimpanzee primate gamma-like retroviruses to chimpanzee since the human-chimpanzee split. The presence of the BaEV-like PtG2 proviruses in *Pan* sp. but not *Homo* sp., and the presence of additional PtG2 LTR BLAST hits in PanTro1 but not in hg16, signifies transmissions after the *Homo-Pan* speciation (Table 2). As noted by van der Kuyl et al., BaEV-like viruses spread among African primates, and probably also to cats, in recent evolutionary time (54–56). In fact, MLV-like gammaretroviruses infected, and occasionally became endogenized in, a number of mammals during this period (11, 37, 49). This is an ongoing process, demonstrated by the widespread polymorphism of MLV integrations among inbred mice (46, 47, 52, 53). The inferred pattern of horizontal spread of the PtG groups is similar to that of simian immunodeficiency virus SIV_{cpz} (3) and human immunodeficiency virus (20), which most likely arose by transfer from smaller primates to chimpanzee and from chimpanzee to human, respectively. Based on the phylogenetic analysis (Fig. S5 in the supplemental material), the PtG2 elements were, like SIV_{cpz} (3), judged to originate from one or several small primates. The relative scarcity of primate sequences prevented the demonstration of a specific transmission route(s) for the PtG1 elements. They were similar to baboon and macaque sequences.

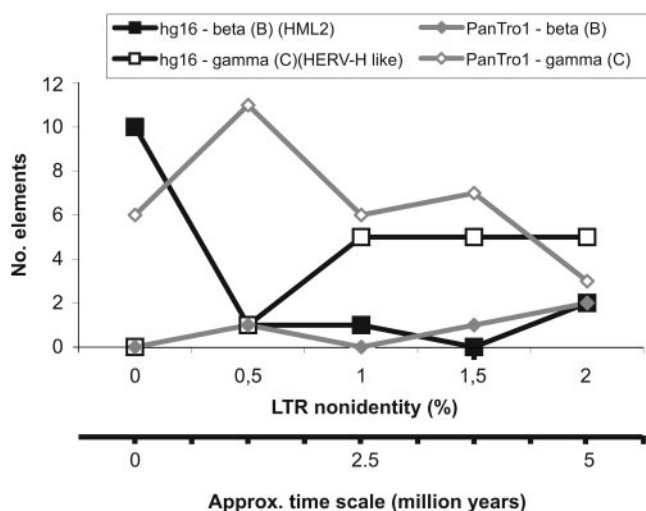


FIG. 2. LTR differences in recently integrated beta-like (HML2 group) and gamma-like ERVs in the human (hg16) and chimpanzee (PanTro1) genomes.

The macaque retroviral similarity was unexpected. African macaques are present in only a small North African population, remote from the ancestral chimpanzee habitats in forests and forested savanna. Consequently, they are unlikely to have been in close contact with the chimpanzees. However, the possibility that macaques and chimpanzees have overlapped geographically in the past should not be excluded. The relatedness of PtG1 to macaque proviral sequences may represent similarity to widely distributed non-ape primate retroviruses. Our observations are thus consistent with the existence of a network of relatively frequent horizontal retroviral transmissions, followed by occasional endogenization, among primates. Exposure of wounds to prey blood during predation or eating of retrovirus-rich placentae (3, 20, 54) are possible explanations. Interspecies retroviral transmission via blood-sucking insects may also occur (58). Finally, it cannot be excluded that retrotransposon DNA may be integrated after uptake from the alimentary canal (19). Chimpanzees frequently eat other primates, like baboons, geladas, and colobi (21, 45), which harbor ERVs similar to the PtG retroviral sequences. The probable cases of horizontal transfer of gammaretroviruses to chimpanzees thus agree with the predatory practices of chimpanzees. It is, however, intriguing that the human genome seems to have been spared from the PtG integrations. Human ancestors and chimpanzees may have been differentially exposed through differing hunting practices (13, 40, 45). Differences in ERV fixation due to population size and distribution could also be reasons.

Differences in recent activity between beta- and gamma-like retroviruses. If LTR divergences for all RetroTector-derived ERVs are used as surrogate markers for integration times, an expansion of a limited number of human HML2 (i.e., hg16-beta) integrations appears to have started around 1.5 to 2 million years ago (approximately 0.5% LTR nonidentities) (Fig. 2). The expansion may still be ongoing, because the curve peaks at 0% LTR divergence. However, LTR-LTR homogenization by gene conversion could lead to falsely low LTR

divergences, precluding an exact interpretation (24, 30). The betaretrovirus-like HML2 is a relatively large HERV group, of which the majority is common to *Homo* and *Pan* spp. (data not shown). A simple explanation for the recent HML2 expansion in humans could be back mutation to replication competence ("breakout"; see below). Even if these recently integrated HML2 elements have been labeled "human specific" (4, 38, 39), the sequence record of primates and other possible contributors of HMLs is incomplete. Cross-species transmissions of HML2 thus cannot be entirely excluded. Figure 2 indicates that the gammaretroviral integrational activity was separate in time from that of betaretroviruses. The recent gammaretroviral integrations of both species differ from the HML2 ones by containing more disrupted genomes yet with low LTR differences (Table 2). Retroviruses may differ substantially in mutation frequency (23, 31, 44). Although the frequency of gene conversion is highly dependent on sequence similarity, the low LTR divergences in otherwise disrupted, and thus probably ancient, gammaretroviral elements can have been caused by LTR homogenization (24, 30).

Complementation in *trans*, where particles formed by more complete ("midwife") proviruses package RNA of less complete ones, as proposed for the HERV-H-like group (28, 36), is another alternative. As expected, the markedly skewed nucleotide distribution of HERV-H elements in general (27) is also present in the recently integrated and disrupted HERV-H elements (Table 2).

Thus, both the human and chimpanzee genomes have been subjected to different kinds of recent retroviral integrations. A BLAT search for LTRs of recent unique ERVs with a stringent criterion (>98% of maximum BLAT score, using either of the 5' and 3' LTRs) resulted in numerous hits (Table 2), but only in the cognate genome. This also applies to the otherwise homogeneous HML2 group. Consequently, in the past, there must have been many more integrations of these elements in the chimpanzee and human genomes than the currently residing ones (Table 2). They may have become looped out through homologous recombination between the LTRs, as postulated previously (48). PCR would be a suitable method to address the amount of solitary LTRs but was out of scope for this study. It is also noteworthy that a BLAT search with LTRs of the selected human HERV-H-like elements resulted in fewer hits (Table 2), suggesting that retroviral RNAs with mutated R, U5, and U3 LTR portions resulted in these integrations, as expected from the "midwife" master model (28, 36). HERV-H probably did not reintegrate recently to the same extent as the HML2 elements did (Fig. 2 and Table 1). It is likely that, for more than 30 million years, the HML2 group multiplied mainly through reinfections rather than *cis* retrotransposition or *trans* complementation (5). The high Pol similarities among the recent HML2 elements (approximately 98% [Fig. S2 in the supplemental material]), together with the low (<2%) LTR divergence, indicate a common origin after *Homo-Pan* speciation. LTR homogenizations by gene conversions are unlikely to have occurred simultaneously in all different HML2 loci (Table 2) after the *Homo-Pan* sp. split, which concurs with the uniqueness of these ERVs in *Homo* sp. The recent expansion of highly related HML2 integrations (Fig. 2) may have derived either (i) from a random mutational activation of a slightly damaged, preexisting HML2 element or (ii) by rein-

fection of humans with HML2 from another species. We currently favor the first hypothesis, since there is no known source of infectious HML2 in animals close to humans or human predecessors. According to the “breakout” hypothesis (7), co-packaged RNA of partially defective ERV elements occasionally may recombine, thereby rescuing and optimizing retroviral function during reinfections from within. HML2 elements are in general the most complete of all HERVs. Old, relatively intact HML2 elements could have assisted in a stochastic fashion in the recent HML2 activation in *Homo* sp. and done so to a lesser extent in *Pan* sp. This possibility should be further investigated.

Conclusion and a caveat. Inevitably, though the screening strategy is likely to detect most young integrations, the genome may contain an undefined portion of older elements where gene conversion caused LTR homogenization. If supplemented with a check for uniqueness (i.e., ERVs occurring in only one or the other species) against the next genome, as conducted here, the approach should correctly detect recent integrations which are unique to humans or chimpanzees. However, a complication is that the assessment of uniqueness for *Pan* versus *Homo* sp. elements is stronger than vice versa due to the poorer *Pan* sp. draft sequence quality. Eventually, this could lead to an erroneous impression of uniqueness for a human sequence. The matching of flanks of the seemingly human-specific HERV-H integrations into the chimpanzee sequence was not convincing. They were therefore not underlined in Table 2. The low number of LTRs with high similarity to the suspected human unique HERV-H proviruses (Table 2) is consistent with the “midwife” master hypothesis (28, 36), because (re)integration-competent proviruses would be more likely to give such single LTRs, while copackaged defective ones would not. Thus, the human unique HERV-H-like sequences (Fig. 1 and Table 2) eventually will need additional experimental analysis. Allelic variation and deletions are additional obstacles. However, precise deletions of proviruses are unlikely to occur (35), especially in the many different loci on different chromosomes presented here. Thus, it is more likely that our observed genomic ERV differences are the results of gain rather than loss. Further, as shown here, LTRs corresponding to the unique proviruses occur frequently but only in the cognate genome. In the BLAT search, they outnumber their proviral counterparts (Table 2). Instead of precise proviral deletions, the higher number of recognized LTRs shows a more likely event of ERV loss through homologous recombination and looped-out proviruses (48). Allelic variation cannot be covered in the single-sequence genome assemblies. A locus-specific PCR, preferably with many individuals, could be used to address this problem but was out of the scope of this study. The false LTR similarity (gene conversion) and false uniqueness problems were addressed by bioinformatic means, as discussed above.

The numerous recent species-unique proviruses, and the larger number of similar species-unique (mainly solitary) LTRs, show that both *Homo* and *Pan* sp. genomes have distinct sets of recently active ERVs. The comparison of retroviral sequences in *Homo* and *Pan* sp. genomes highlights the importance of (i) habitat, interspecies contact, and predator-prey relations facilitating cross-species retroviral infection from “outside” and/or (ii) probable stochastic reactivation of preex-

isting ERVs followed by reinfection from “inside” as determinants of the retroviral genetic setup of a species.

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