博士論文

(Distribution profiles of human endogenous retroviral LTRs in human chroomosomes)
(ヒト染色体中におけるヒト内在性レトロウイルスの分布様式)

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# Distribution Profiles of Human Endogenous Retroviral LTRs in Human Chromosomes

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ABSTRACT

The human genome carries multiple copies of sequences related to endogenous retroviral genomes. We investigated the distribution of the multicopy type endogenous retroviral sequences, HERV-A and RTVL-H2, in twenty-four human chromosomes by Southern analyses using DNA from flow sorted chromosomes or rodent cells carrying a single human chromosome. The results showed that HERV-A and RTVL-H2 are distributed among all human chromosomes and that each chromosome has specific Southern blot profiles. The chromosome-specific pattern did not show polymorphism, except in few cases, when the same chromosome obtained from different individuals was compared.

These chromosome specific Southern hybridization profiles may be useful for chromosome karyotyping. This would allow the integrity of human chromosomes in human-rodent somatic cell hybrids to be monitored without using conventional cytogenetic methods.

# INTRODUCTION

The human genome contains a variety of sequences which are related to endogenous retroviruses. This endogenous retrovirus is thought to be categorized in retroposons which also exist in human genome at high frequency.

Retroposon is a troansposon which is integrated in genome through RNA intermediates. In this process, transcribed retroposons go against the Central Dogma, that is to say, integrate its active RNA information into genome again using reversetranscriptional mechanisms and increase its number in genome.

Retroposon is classified into Viral Superfamily and Non viral Superfamily by Weiner (Weiner et al., 1986). The former family includes retrovirus and retrotransposons which contains viral genes. Viral genes code for the enzymes which are neccessaly for its retropositions, such as reversetranscriptase or integrase. The latter family is the sequences thought to be integrated in genome through reversetranscription. This family includes the many copies of processed pseudogenes.

In the Viral Superfamily, endogenous retrovirus is categolized. Retrovirus that cause tumors in chickens were among the first animal viruses to be described. Its life cycle depends on unusual biochemical reactions, most obviously the reverse transcription of genomic RNA into DNA and the orderly integration of viral DNA into host chromosomes to form provirus. Retrovirus have been found in all vertebrate animals including fish, birds, rodents, and non-human primates.

Recently in human, human immuno differency virus (HIV) which causes the acquired immunodifferency syndrome (AIDS), and adult human T cell leukemia virus (HTLV-I) were discovered and the relationships between these retroviruses and human genome have been investigated.

The structures of endogenous retroviruses are very much like a provirus form of exogenous retrovirus. But no endogenous retrovirus sequences which contain intact viral genes have been reported for now. So almost all of

endogenous retroviruses in genome may not have properties of infectious exogenous retroviruses. All normal human genomes contain the endogenous retroviruses and these sequences are inherited to decendants through germ lines.

The total copy number has not been determined yet, but in the case of rodent it is said that the ratio is about 1% of its genome. The origin is thought to be the exogenous infecting retrovirus. Very little is known about the function. In some cancer cell lines or in early embryonic stage of mouse, remarkable increases of endogenous retrovirus transcriptions have been reported, but the distinct function has not been resolved now. The detailed loci on chromosomes and the possible extstence of target sequences or target chromosomes are unknown. If the distribution profiles of endogenous retroviruses in chromosomes are investigated and understood, the effects of endogenous retrovirus on other genomic genes, how they have accumulated in the human genome may be solved.

I have initiated studies on distribution of the human endogenous retrovirus sequences in human chromosomes taking HERV-A (Emi et al., 1988) and RTVL-H2 (Mager et al., 1987) as examples. Currently available data show that there are at least 12 families (Noda et al., 1982: O' Connel et al., 1984; Callahan et al., 1985; Maeda et al., 1985; Paulson et al., 1985; Repaske et al., 1985; Deen et al., 1986; Leib-Mosch et al., 1986; May et al., 1986; Ono et al., 1986; Harada et al., 1987; Kröger et al., 1987; Mager et al., 1987; Silver et al., 1987; Emi et al., 1988). In these 12 families, these two endogenous retroviruses are representative multicopy type endogenous retroviruses. These two sequences do not cross hybridize with each other. HERV-A was found in upstream regulatory region of salivary type $\alpha$-amylase gene in chromosome 1 and RTVL-H2 was found in the region promixmal to the human $\beta$-globin. We addressed the question as to whether the integration sites within different chromosomes is selected at random. For this purpose, I extended our previous limited observations on the distribution profiles of the HERV-A LTR sequence with some human chromosomes (Nakamura et al., 1991), and obtained Southern patterns of the HERV-A LTR

sequence, as well as RTVL-H2 sequence from all human chromosomes. The results show the uneven distribution of LTR copies among different chromosomes, specific integration sites were not indicated. The distribution patterns in each human chromosome are unique, with limited polymorphisms. These observations show that these endogenous retroviruses widely spread in all human genome. But the integration sites seem to be preserved over the different individuals. The chromosome-specific profiles were found as useful indexes for monitoring human chromosomes in somatic hybrid cells that are widely used for mapping genes and for making chromosome-specific DNA libraries.

## MATERIALS AND METHODS

*Somatic hybrid cell lines*

To investigate the properties of endogenous retrovirus among different human chromosomes, a pannel of monochromosomal or subchromosomal somatic cell hybrids was collected as shown in Table 1. A series of microcell hybrids, termed A9 (Neo-x)-y, have been reported elsewhere (Koi et al., 1989). The monochromosomal property was confirmed by G-banding patterns.

GM hybrids with prefix GM were purchased from the NIH Human Genetic Mutant Cell Repository at the Coriell Institute (Camden, New Jersy). Many of the hybrids used have been previously published, and references to the original construstion, growth conditions and cytogenetic characterization are provided in Table 1.

*Preparation of sorted chromosomes using a fluorescence-activated flow sorter*

I separated and collected the human chromosomes 6, 9, 13, 18, 19, 20, 21, 22 and Y respectively using dual laser system fluoresense-activated flow sorter. Cells were cultured to subconfluence in RPMI1640 medium with 10% FCS, or MEM-$\alpha$ medium with 10% FCS, and further incubated for 18 hrs in the presence of 0.2 $\mu$g/ml of colcemid. Metaphase chromosomes were collected using polyamine-digitonin, then size-fractionated by sedimentation through a 5 to 30% sucrose gradient, according to Fukushige et al (1986). The fractionated chromosomes were dually stained with Hoechst 33258 (2.0 $\mu$g/ml) and Chromomycin $A_3$ (50 $\mu$g/ml) in the presence of 10 mM $MgCl_2$ and processed through a fluorescence-activated flow sorter, CHROSS-I (Japan Spectroscopic Co, LTD). Laser beams excited the dyes under the following conditions, Hoechst 33258, 355nm, 300mw, Chromoymsin $A_3$, 458nm 250mw. About $4\times10^6$ of each chromosome were prepared. Chromosomes 18, 19, 20, 21 and 22 were sorted from GM0131, and chromosomes 6, 13 and Y were from UV21HL4. Each flow

Table 1 Rodent-human hybrid cell lines carrying a single human chromosome

| Hybrid[1)] | Repository number[2)] | Human chromosome | Rodent parent | Reference |
|---|---|---|---|---|
| A9 (Neo-1)-4 | | 1 | A9 | 13. |
| A9 (neo-2)-1 | | 2 | A9 | 13. |
| 314-16 | GM10253 | 3 | CHO | 29. |
| HHW416 | GM10115 | 4 | CHO | 3. |
| A9 (Neo-5)-4 | | 5 | A9 | 13. |
| BG15-6 | | 5 | CHO | 11. |
| HHW105 | GM10114 | 5 | CHO | 4. |
| A9 (Neo-6)-3 | | 6 | A9 | 13. |
| A9 (Neo-7)-2 | | 7 | A9 | 13. |
| A9 (Neo-8)-1 | | 8 | A9 | 13. |
| PK-87-9 | GM10611 | 9 | CHO | 38. |
| A9 (Neo-10)-3 | | 10 | A9 | 13. |
| A9 (Neo-11)-1 | | 11 | A9 | 13. |
| J1 | | 11 | CHO-K1 | 12. |
| A9 (Neo-12)-4 | | 12 | A9 | 14. |
| HDm-5 | GM10479 | 14, (16) | 3T6 | 18. |
| A9 (Neo-15)-2 | | 15 | A9 | 13. |
| CY18 | GM10567 | 16 | A9 | 1. |
| MH22-6 | GM10498 | 17 | LTMK | 35. |
| A9 (Neo-18)-5 | | 18 | A9 | 13. |
| A9 (Neo-19)-1 | | 19 | A9 | 13 |
| 5HL9-4 | GM10449 | 19 | CHO | 34. |
| PK-87-19 | GM10612 | 19 | CHO | 38. |
| A9 (Neo-20)-3 | | 20 | A9 | 13. |
| WAV17 | GM08854 | 21 | A9 | 32. |
| | GM6853A | X | 7777-14b-AZA | |
| | GM6318B | X | CH65091 | |
| | GM7317 | Y | CHW1103 | |
| UV41HL4 | | 6, 9, 13, 16, 18, Y | CHO | 8. |
| BG15-9 | | 5pter→q23 | A9 | 11. |
| BG15-7 | | 5pter→q22 | A9 | 11. |

1) Hybrid cells A9 (Neo-x)-y have been described by Koi et al. (13) The monochromosomal property was confirmed by G-banding patterns.

BG15-6 was obtained from Y. Kaneda (Inst. Mol. Cell Biology, Osaka University).

J1 was obtained via Y. Nakamura (Japan Foundation for Cancer Research), through permission of D. Hausmann (Massachusetts Institute of Technology).

2) GM series refer to the reference numbers of NIH Human Genetic Mutant Cell Repository (Camden, N. J. ).

3) Chromosomes shown in parentheses ( ) are present in <20% of cell analyzed.

karyotypes were shown in Fig. 1. The purity of the chromosomes, monitored by re-staining and running through the CHROSS-I, was over 95%. The chromosal DNAs were then extracted by phenol.

*Southern analysis*

High molecular DNAs were extracted from the cells or sorted chromosomes according to the method described by Maniatis et al. (1982).

DNAs were completely digested with EcoRI, electrophoresed in 0.6% agarose, then transferred to a Nylon membrane (Hybond N+). The filters were prehybridized at 65°C for about 3 hours in 5×SSPE (1×SSPE : 0.18M NaCl, 0.1M Sodium phosphate and 1mM $Na_2EDTA$) containing 1% SDS, 5×Denhardt's solution (50×Denhardt's reagent : 1% Ficoll (Type 400, Pharmacia), 1% polyvinylpyrrolidone, 1% bovine serum albumin (Fraction V ; Sigma)) and denatured salmon sperm DNA (100 $\mu$g/ml), then incubated at 65°C overnight in the same buffer containing $^{32}$P-labeled LTR probe ($10^6$ cpm/ml). The membranes were washed twice with 2×SSPE containing 0.1% SDS at 65°C for 10 min, followed by 1×SSPE containing 0.1% SDS at 65°C for 10 min, then by 0.1×SSPE containing 0.1% SDS at 65°C for 15 min. The membranes were then autoradiographed at −70°C using Kodak XAR-5 film with an intensifying screen.

The HERV-A probe was a 371 bp Hind Ⅲ-BstN Ⅰ fragment containing the major portion of the 430 bp HERV-A 5' LTR (Emi et al., 1988) (Fig. 2A). The RTVL-H2 probe used was a 390 bp StuI and Hind Ⅲ fragment containing the major portion of the 450 bp 5' LTR (Mager et al., 1987), kindly provided by Dr. D. L.Mager. (Fig. 2B).

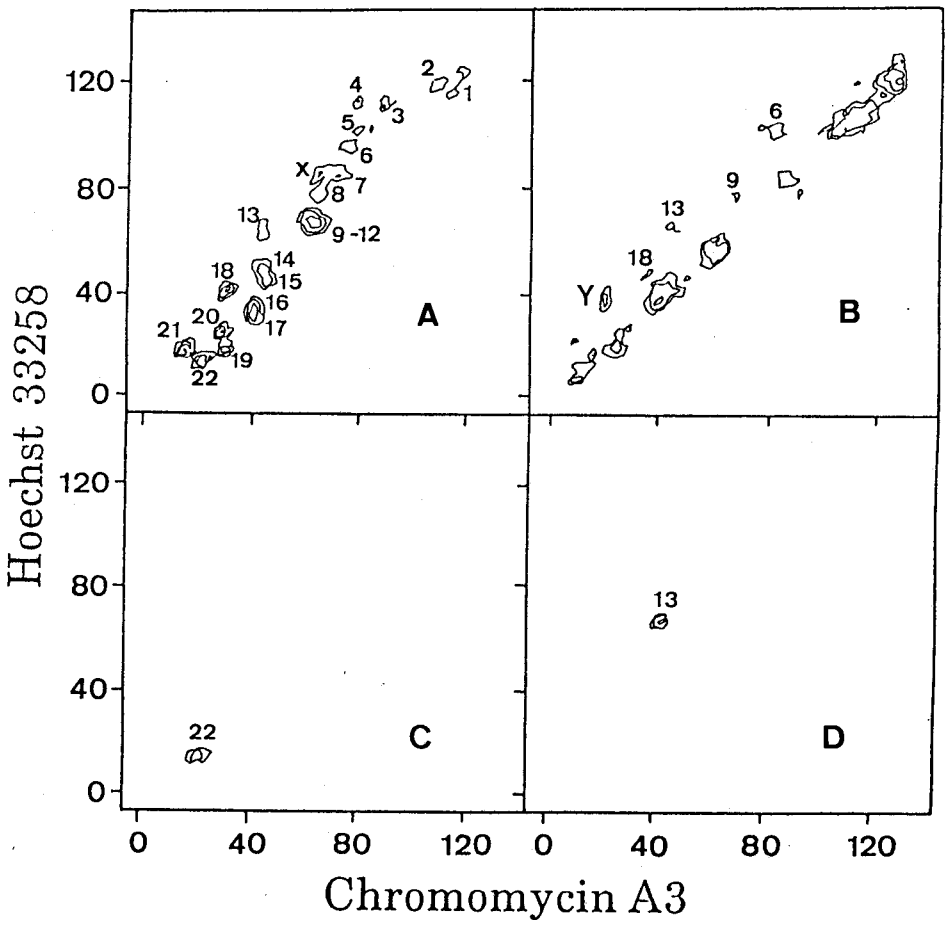


Fig. 1. Flow karyotypes of human lymphoblastoid line GM131(A) and human-rodent hybrid cell line UV41HL4(B). (C) and (D) are resorted patterns of #22 and #13 respectively.

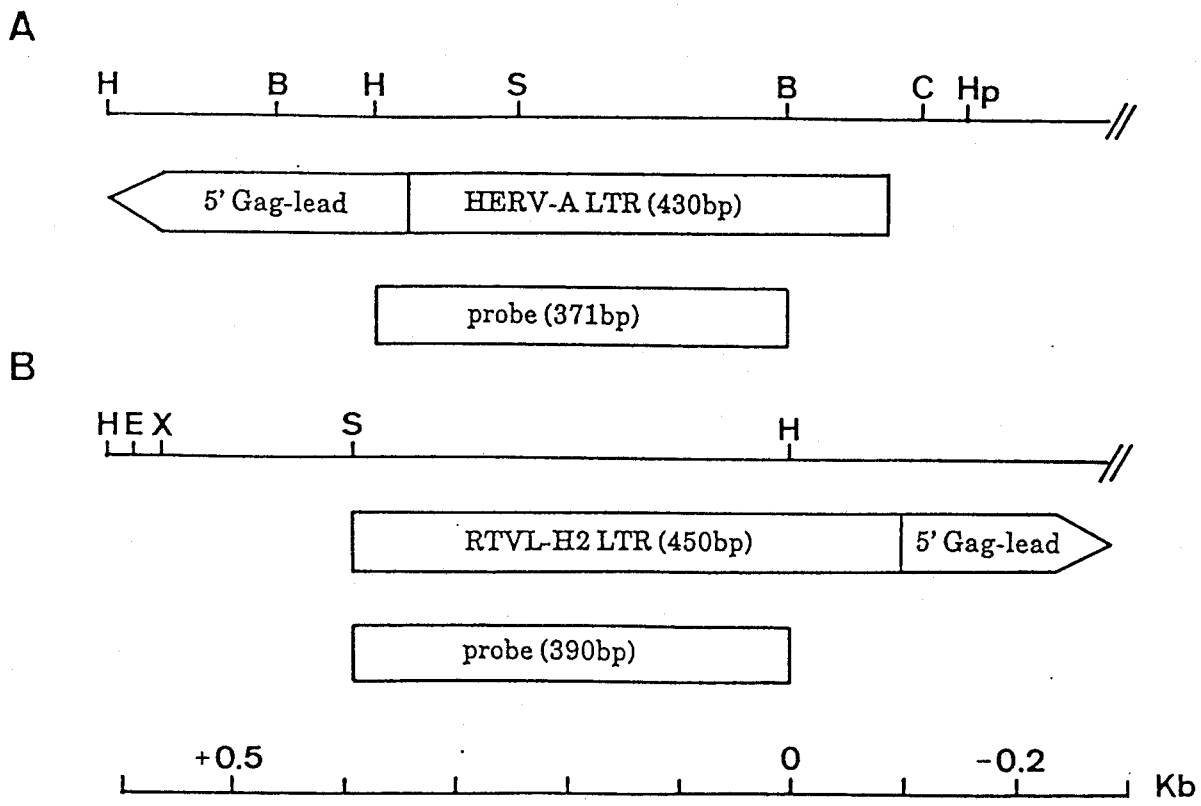


Fig. 2. LTR probes of HERV-A (A) and RTVL-H2 (B) used in this study. The restiction sites are : B, Bam HI; C, Hinc II; E, *Eco*RI; H, Hind III; Hp, HpaI; S, StuI; X, XbaI.

## RESULTS

*Genomic Southern hybridization profiles using HERV-A LTR and RTVL-H2 LTR probes.*

To determine whether or not these endogenous retroviruses' integration sites are conserved among different individuals, we compared banding patterns of EcoRI digested Southern hybridiation profiles using LTR probes of each endogenous retroviruses.

As described in method section, I used a 371 bp and 390 bp LTR sequences as a probes for hybridization (Fig. 2A, B).

First, genomic DNA from human leukocytes was extracted, cleaved by EcoRI that does not cleave within the HERV-A LTR and RTVL-H2 LTR, then analyzed by Southern blotting using each LTR DNAs as probes. These hybridization profiles resulted in many hybridizing bands. Next, I examined DNAs from 30 unrelated individuals. No apparent polymorphisms were detected with the main bands. These 30 unrelated individuals included Cauasians, American Black (Hela), Asian and both sexes. Some of these profiles are shown in Fig. 3 and Fig. 4. I tried to compare the HindⅢ Southern hybridization banding patterns using each LTR probes (data not shown). In these HindⅢ restriction patterns, no apparent polymorphisms were detected in 30 unrelated individuals.

The agreement of these endogenous retroviruses' restriction patterns suggests that integration loci on chromosomes are common among individuals. The band intesity may reflect the presence of multiple copies in a fragment, or overlapping.

*Banding patterns of HERV-A LTR in human chromosomes.*

It is very interesting to know whetehr or not these endogenous retroviruses accumulate in some specific chromosomes. To investigate the distributions of these endogenous retroviruses among each number of human chromosomes , I

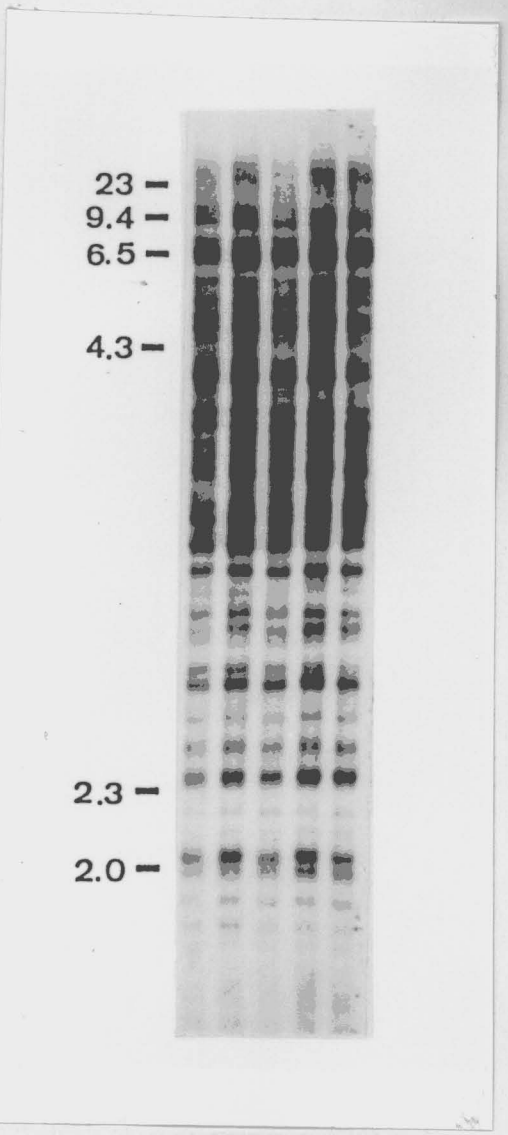


Fig. 3. Genomic Southern hybridization profiles from five individuals using the HERV-A LTR probe. 1 and 2, normal skin fibroblasts; 3, lymphoblastoid cells; 4, leukocytes; 5, hepatocytes. Genomic DNAs were digested with *Eco*RI. Numbers at the left side of each panel show sizes (kb) of the DNA.

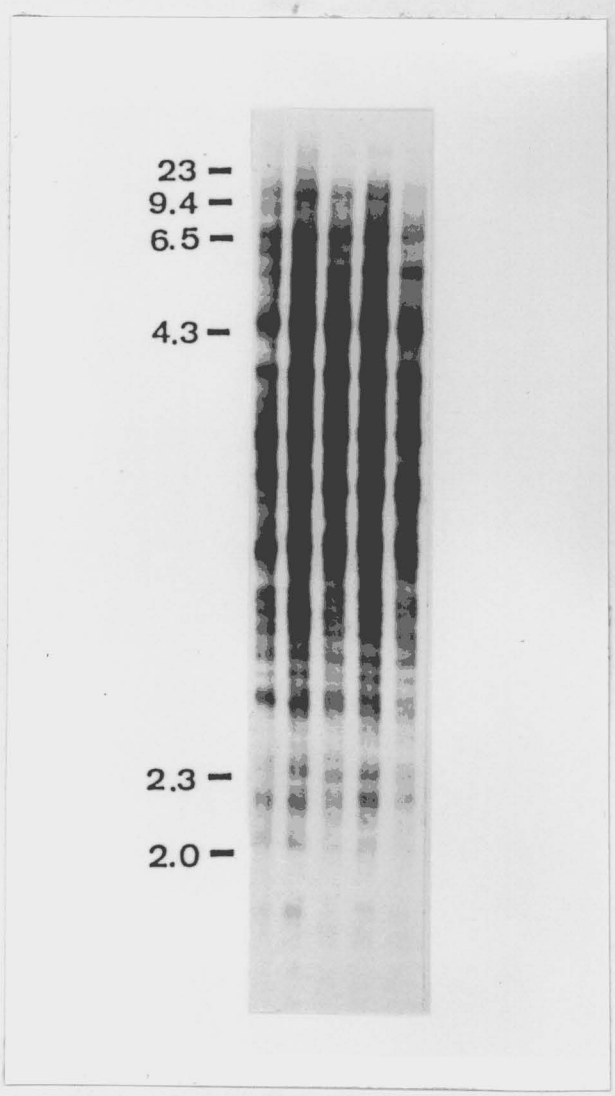


Fig. 4. Genomic Southern hybridization profiles from five individuals using the RTVL-H2 LTR probe. 1 and 2, normal skin fibroblasts; 3, lymphoblastoid cells; 4, keukocytes; 5, hepatocytes. Genomic DNAs were digested with *Eco*RI. Numbers at the left side of each panel show sizes (kb) of the DNA.

collected a set of monochromosomal hybrid cells that carry one human chromosome in a background of rodent chromosomes. Details of these hybrids are summarized in Table 1. DNA from each hybrid cell was extracted, cleaved by EcoRI and then analysed by Southern blotting using HERV-A LTR DNA as the probe. Because HERV-A LTR does not cross-hybridize with retroviral sequences in rodents, Southern profiles of the genomic DNA from monochromosomal hybrid cell represents that of the unique human chromosome. DNAs from chromosomes 6, 13, 18, 19, 20, 21, 22 and Y were prepared from sorted chromosomes. Details of this operation are described in Methods section.

The results are collectively shown in Fig. 5. The HERV-A LTR and its related sequences are distributed widely among all the human chromosomes, and the banding pattern of each chromosome is chromosome-specific. The number of bands in each chromosome does not seem to be proportional to the size of the chromosome, suggesting that integration did not take place by randomly selecting the sites. However, from the currently available data, nothing more can be concluded. Variation in band intensities may reflect differences in the number of repeat units that carry the HERV-A sequence (see below).

*Comparisons of HERV-A LTR banding patterns of a chromosome with that of the same chromsome obtained from different sources.*

To determine whether or not these banding patterns are conserved among different individuals, we compared banding patterns obtained from chromosomes that have been prepared from different sources. Chromosomes 5, 6, 11, 18, 19, 20, 21, X and Y were chosen for this purpose. The results are collectively displayed in Fig. 6.

With chromosome 5, where DNAs from 3 independent monochromosomal hybrid cell lines A9 (Neo-5)-4, HHW105 and BG15-6 were compared, 11 major bands were found in common, and three bands were missing in HHW105 (arrows). Studies with deletion hybrids have demonstrated that these three

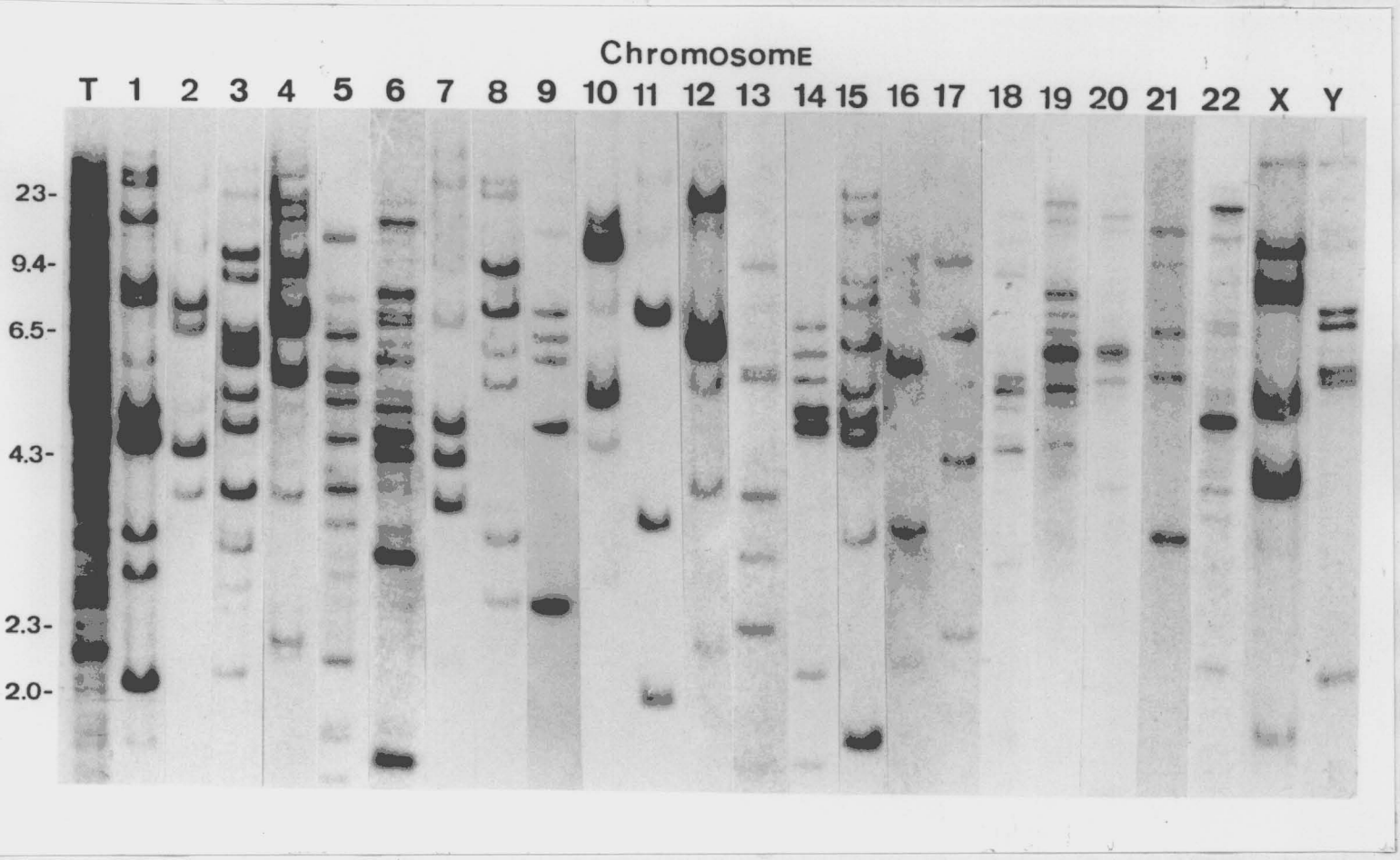


Fig. 5. Banding patterns of HERV-A LTR and its related sequences among the twenty four human chromosomal DNA's cleaved by EcoRI. Numbers at the top of each lane represent the chromosome number. Numbers on the left hand side of panel show size (Kb). Chromosomes 6, 9, 13, 18, 19, 20, 21 and Y were purified using a fluorescence-activated cell sorter (see Material and Methods). Other samples were from human-rodent monochromosome hybrid cell lines (Table 1). T is a total genomic DNA from the human nomal lymphoblastoid cell line GM0131. Data regarding chromosomes 5, 11, and X were obtained using A9 (Neo-5)-4, A9 (Neo-11)-1, and GM6853A, respectively.

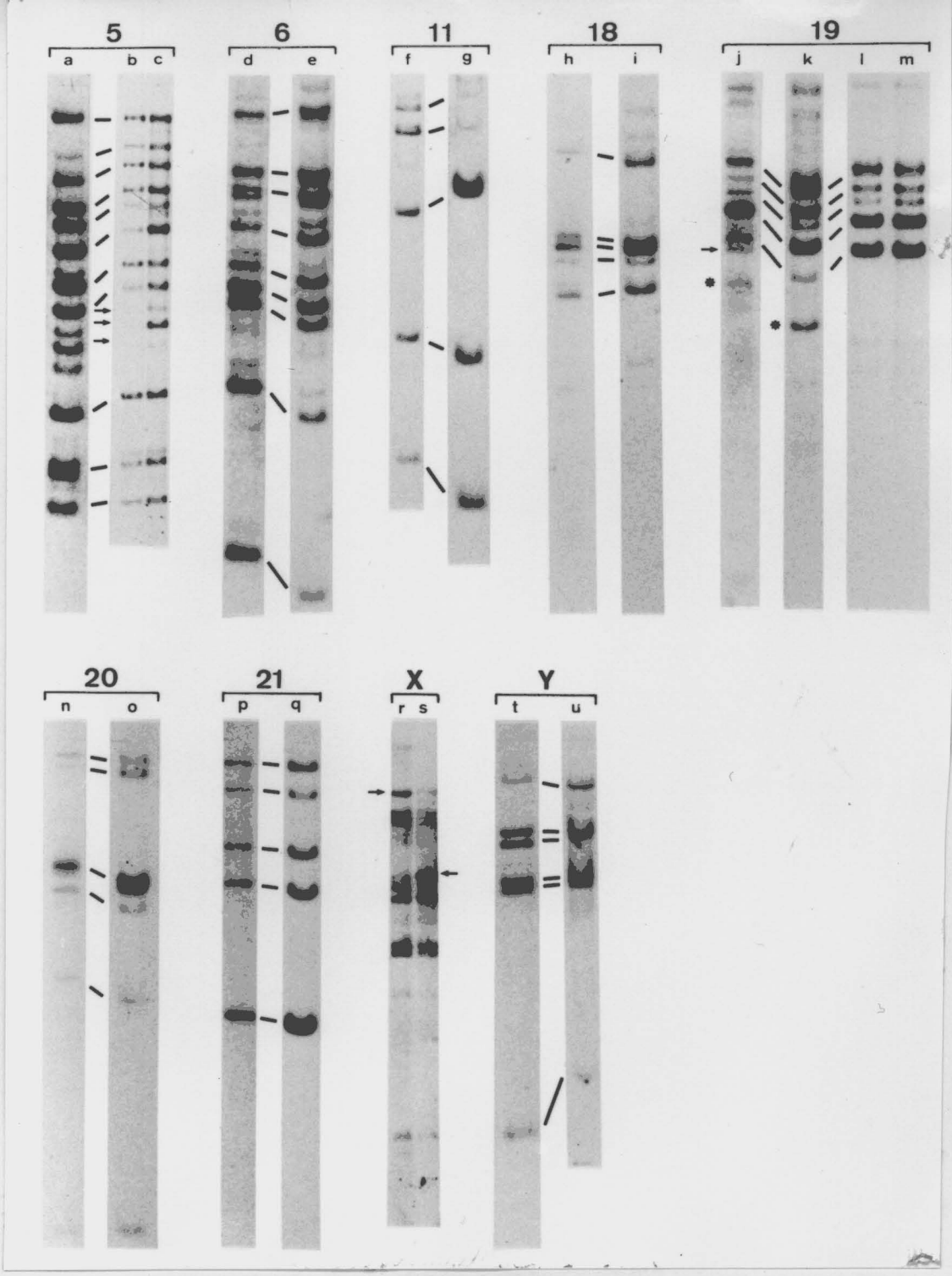


Fig. 6. Comparison of HERV-A LTR banding patterns with the same human chromosome obtained from different sources. Genomic DNAs from different monochromosomal hybrid cells or DNAs from purified chromosomes prepared by a fluorescence-activated cell sorter were analysed by Southern blotting as in the legend to Fig. 5. a, A9 (Neo-6)-3, b, HHW 105, c, BG15-6, d, flow sorted chromosome 6, e, A9 (Neo-5)-4, f, A9 (neo-11)-1, g, J1, h, flow sorted chromosome 18, i, A9 (Neo-18)-5, j, flow sorted chromosome 19, k, A9 (Neo-19)-1, 1, 5HL9-4, m, PK-87-19, n, flow sorted chromosome 20, o, A9 (Neo-20)-3, p, flow sorted chromosome 21, q, WAV17, r, GM6853A, s, GM6318B, t, flow sorted chromosome Y, u, GM6317. The non-matching bands are marked by arrows or asterisks.

bands are located in the distal region in the long arm of chromosome 5 (see below). It is likely that chromosome 5 in HHW105 has an undetected deletion.

With chromosomes 6, 11, 18, 20, 21 and Y, wherein a pairwise comparisons were made with the same chromosomes prepared from different cell lines, no differences were detected. With chromosomes 19 and X, major bands in each set were observed in common, but there were some differences. Four samples of chromosome 19 were compared, and 5 bands were observed in common. However, a band that is not present in lanes l and m can be seen in lanes j and k (asterisks), and lane l displays one extra band (arrow). Although the number of samples we analysed is limited, these differences are probably due to polymorphism. With chromosome X, there are different intensities of two bands (arrows). Because each of these bands differ only in intensity, as compared with the corresponding band in another sample, this may reflect the differences in number of copies that carry the HERV-A sequence. Despite these minor differences, the majority of the bands completely match, indicating that the HERV-A distribution in chromosomes is conserved in most cases, and it is not not highly polymorphic.

*Banding patterns of RTVL-H2 LTR's in human chromosomes*

As noted in the introduction, the human genome carries at least 12 different endogenous retrovirus-related sequences. We extended our observations to an another LTR sequence, RTVL-H2 (Mager et al., 1987), which had been found in the region proximal to the human $\beta$-globin gene (Mager et al., 1984). This LTR is also not cleaved by EcoRI. Approximately 1,000 copies of sequences related to this element have been reported per haploid human genome.

The Southern profiles using RTVL-H2 as a probe with DNAs from individual chromosomes are collectively shown in Fig. 7. Again, chromosome specific distribution profiles were detected. Approximately three to four times as many bands were detected, but their numbers seem to be independent of chromosome size, as were the cases with HERV-A. The number of bands in each

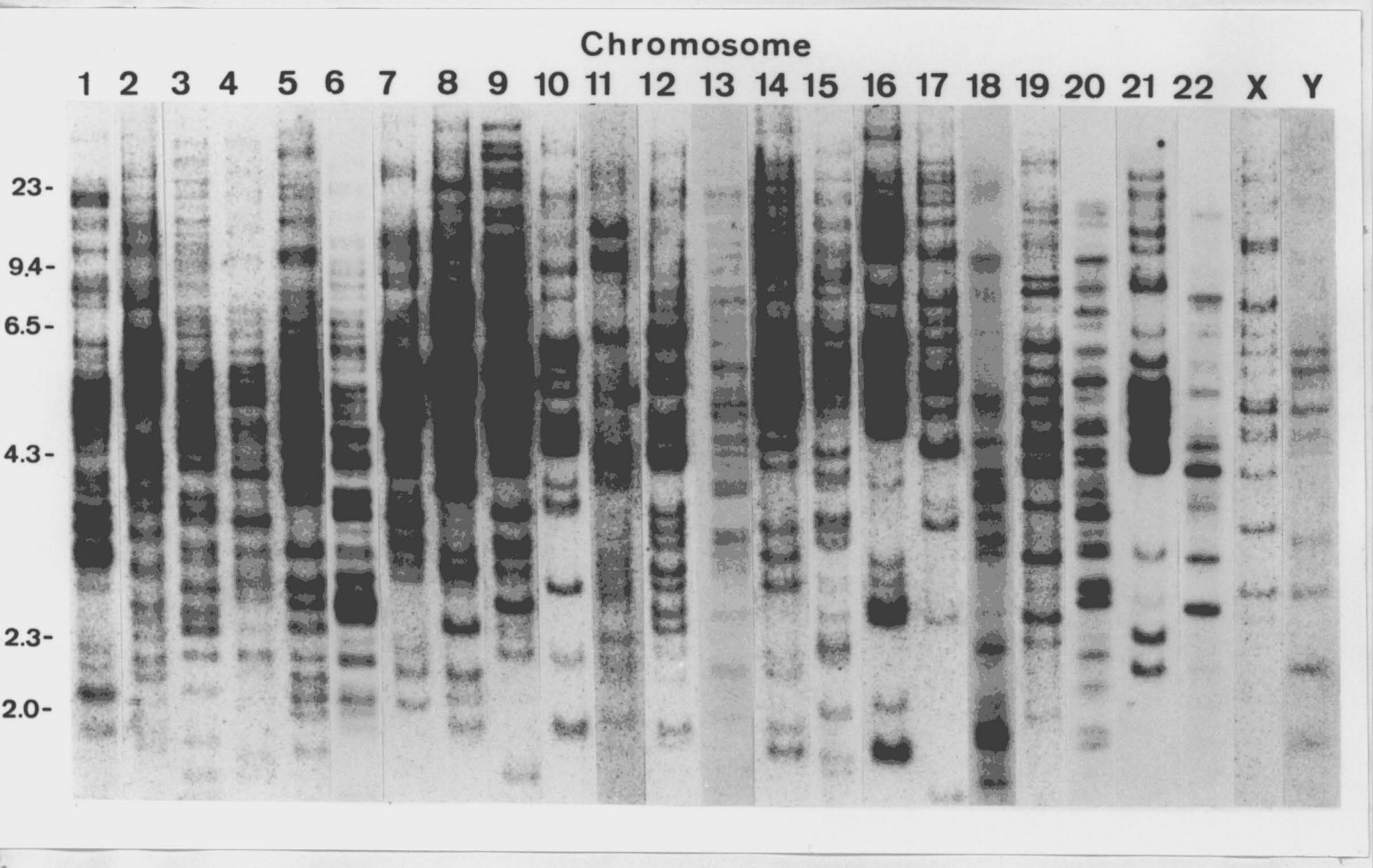


Fig. 7. Banding patterns of RTVL-H2 LTR and its related sequences among the twenty four human chromosomal DNAs cleaved by EcoRI. DNAs of chromosomes 6, 9, 13, 18, 20, 22 and Y were obtained from purified chromosomes using a fluorescence-activated cell sorter. Others were obtained with genomic DNAs from monochromosomal hybrid cell lines. Banding patterns of chromosome 5, 11 and X were obtained with DNA from the same set of hybrid cell lines used in Fig. 5.

chromosome does not seem to be in parallel between HERV-A and RTLV-H2. From these results we can convince that in human chromosomes, there are no preferred target chromosomes for retrovirus integration.

*Comparisons of RTVL-H2 banding patterns in the same chromosome obtained from different sources*

We compared the banding patterns between identical chromosomes obtained from different sources. Chromosomes 5, 6, 18, 19 and 20 were studied, and the results are shown in Fig. 8.

We compared HHW 105 and A9(Neo-5)-4 in chromosome 5, and found that some bands were missing in HHW 105 (marked by asterisks). This observation is in agreement with that in Fig. 6 where deletion was suggested, using the HERV-A probe. Some polymorphisms were also detected (arrows).

In chromosomes 6, 11, 18, 19 and 20, there are almost identical band profiles between the pairs, except for some non-matching bands (arrows). They may be as polymorphic bands as were those with HERV-A.

*Distribution profiles of HERV-A and RTVL-H2 in chromosome 5*

To further examine the locations of LTR sequences within a human chromosome and to determine whether or not these endogenous retroviruses exist in chromosomal specific loci such as telomere or centromer, I compared the banding patterns of the DNAs from the hybrid cell lines BG15-6, BG15-9 and BG15-7, the latter two of which carry only a portion of human chromosome 5 (They are shown in the right figures of Fig. 9 and Fig. 10). The common bands among these samples must have originated from 5pter→q22, carried by the partial chromosome in BG15-7. The results are shown in Fig. 9 and Fig. 10. In the case of HERV-A, 4 bands (arrows) were assigned to 5q31→qter and 2 bands (asterisks) to 5q23 (Fig.9) and in the case of RTVL-H2, 3 bands (arrows) were assigned to 5q31→qter and 3 bands (asterisks) to 5q23 (Fig. 10). These

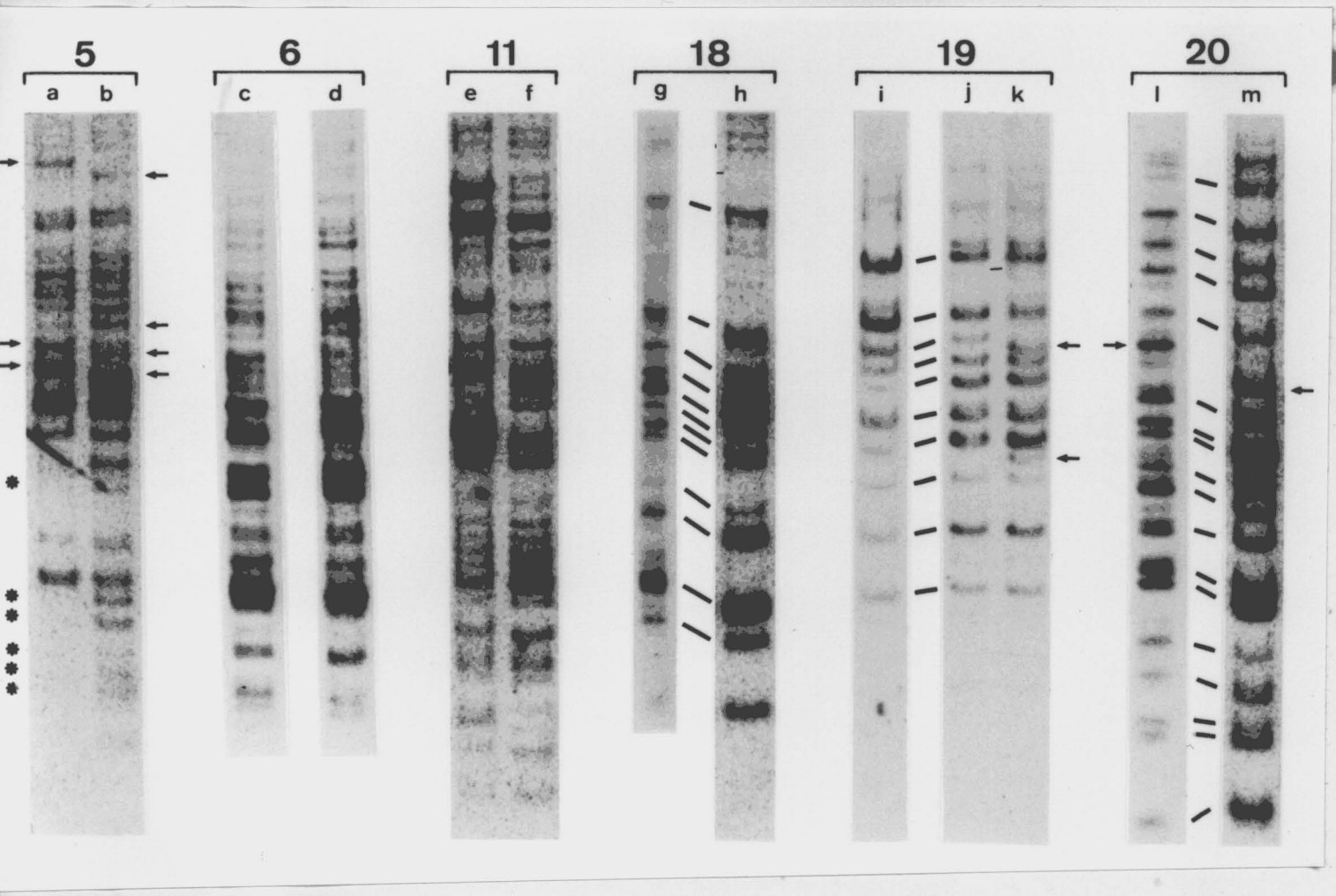


Fig. 8. Comparison of RTVL-H2 LTR banding patterns with the same human chromosome obtained from different sources. Genomic DNAs from different monochromosomal hybrid cells or DNAs from purified chromosomes prepared by a fluorescence-activated cell sorter were analysed by Southern blotting as in legend to Fig. 5. **a**, HHW105, **b**, A9 (Neo-5)-4, **c**, flow sorted chromosome 6, **d**, A9 (Neo-6)-3, **e**, A9 (Neo-11)-1, **f**, J1, **g**, flow sorted chromosome 18, **h**, A9 (Neo-18)-5, **i**, A9 (Neo-19)-1, **j**, 5HL9-4, **k**, PK-87-19, **l**, flow sorted chromosome 20, **m**, A9 (Neo-20)-3.

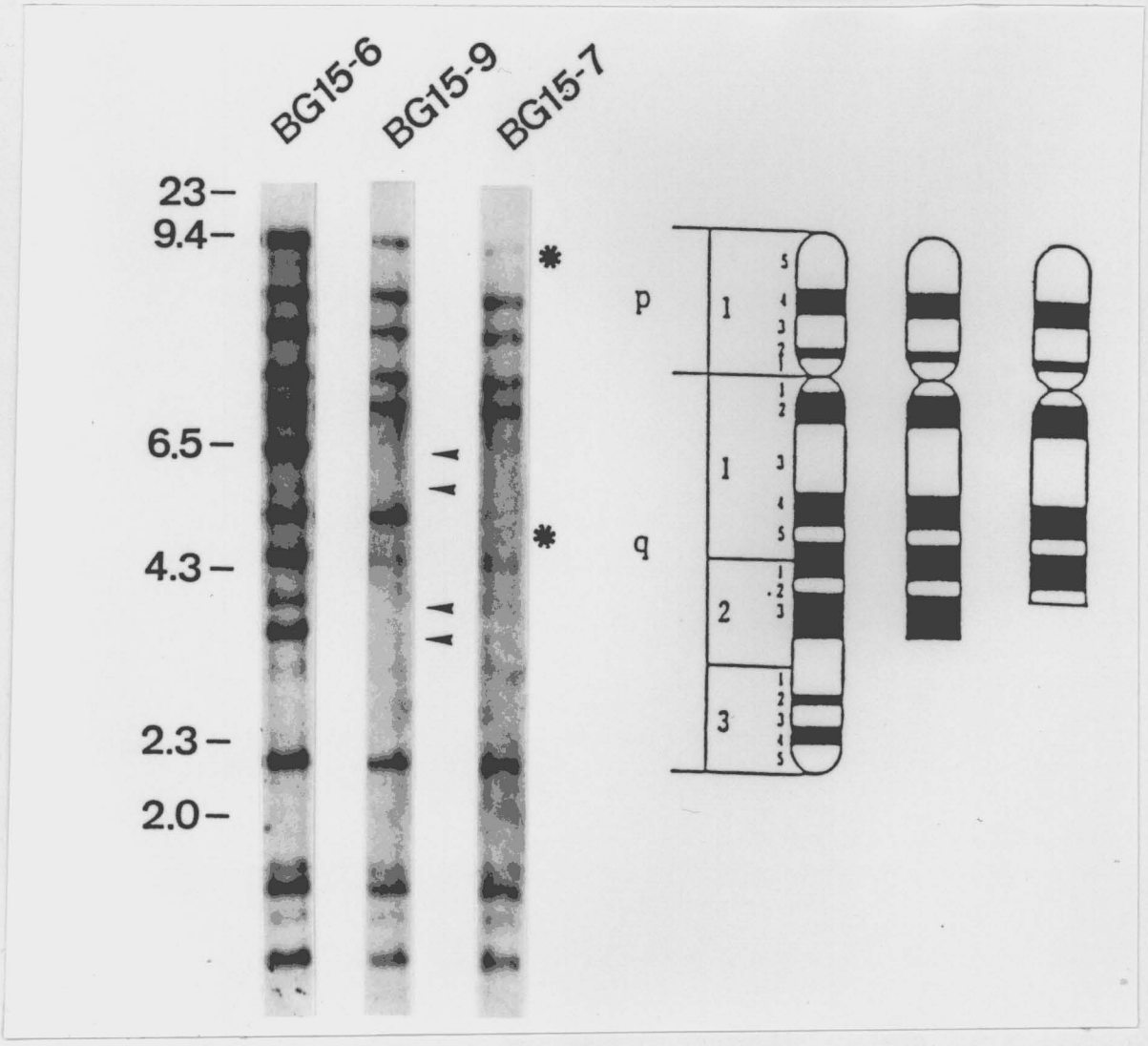


Fig. 9. Assignment of four bands (arrows) to 5q31→qter and two bands (asterisks) to 5q23 by comparing Southern hybridization profiles of EcoRI-digested DNAs from three cell lines carrying the whole or a part of human chromosome 5. The cell lines and the human chromosomes carried are described in Table I.

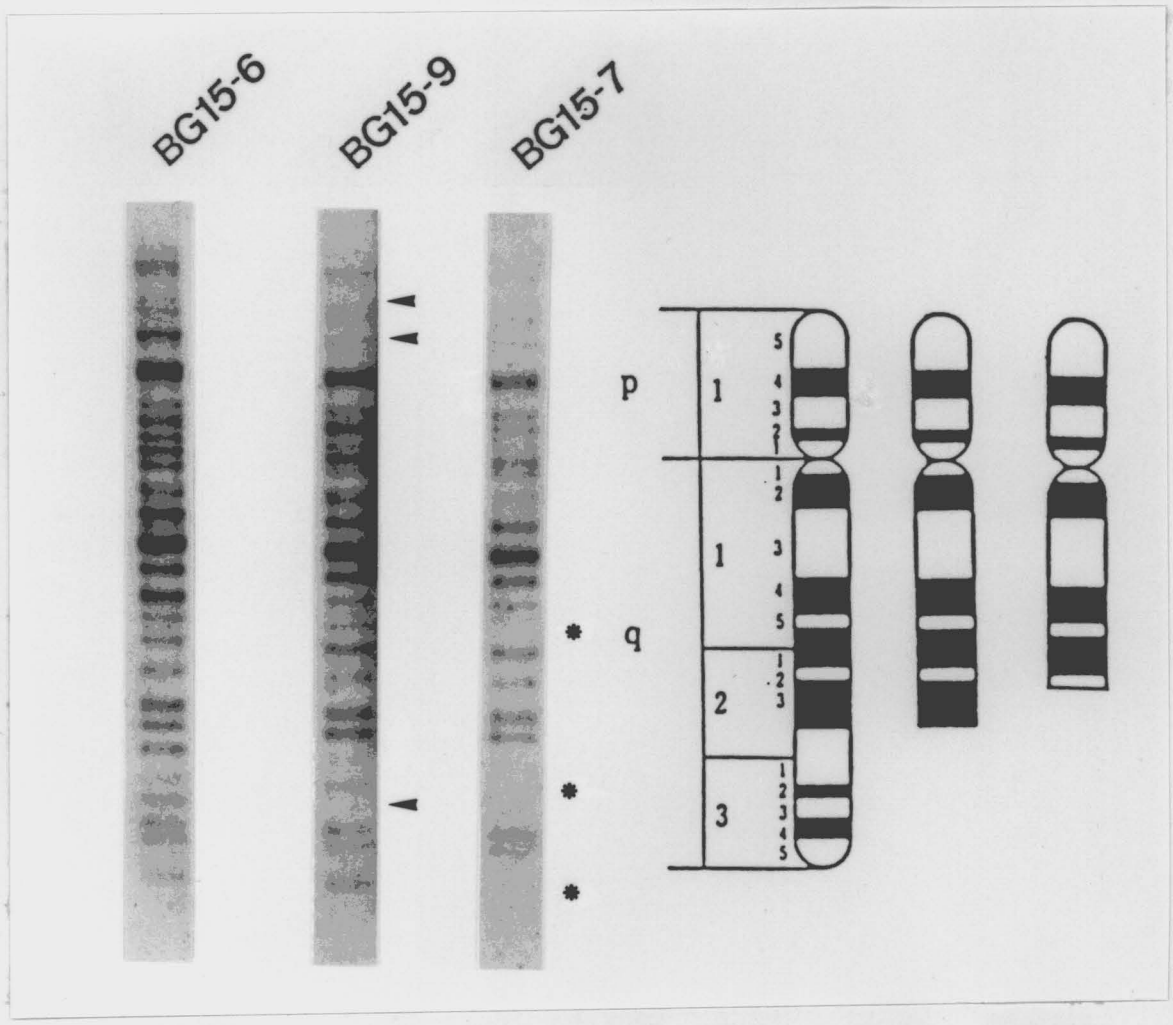


Fig. 10. Assignment of three bands (arrows) to 5q31→qter and three bands (asterisks) to 5q23 by comparing Southern hybridization profiles of EcoRI-digested DNAs from three cell lines carrying the whole or a part of human chromosome 5. The cell lines and the human chromosomes carried are described in Table I.

observation demonstrates not only that the sequences hybridizing with these human endogenous retrovirus LTR are at various sites along the chromosome, but also that they can be sublocalized to specific loci on the chromosome, when a set of deletions is used for the test.

## DISCUSSION

Southern analyses show that the human endogenous retroviruses, HERV-A and RTVL-H2, are widely distributed among all of twenty-four human chromosomes. The number of integrants are not proportional to the size of each chromosome, and the relative number of the integrants are not in parallel between the two endogenous retroviruses. The bias in distribution suggests bias in the selection of integration sites by these sequences, but there are no positive observations to support this idea. In some cases, we compared patterns with a chromosome originating from two or more different sources. The results showed that these endogenous retrovirus are, in general, conserved among different individuals. The conservation of the majority of the bands implies that these endogenous retroviruses exist in conserved regions of the chromosomes, or that they have spread within genome of our early ancestors. Interestingly, some Alu and L1 genes, which are also retrotransposons and which are increasing its number during the evolution in primate genome reside in conserved regions. These intriguing questions related to the participation of retrotransposons in the organization of the human genome (Korenberg et al., 1988) can be solved by collecting more data on integration sites. It can lead to insights into mechanism of retrotranspositions in the genome. However, of about 500 bands with HERV-A, and about 1,500 bands with RTVL-H2, at least 4 and 11 polymorphic bands were detected by pair-wise comparisons with chromosomes 5, 6, 11, 18, 19, 20, 21,X and Y. Some of these polymorphisms may reflect RFLP, as judged from the displacement of a band between two samples. Others might reflect a difference in number of repeat units, because two samples gave rise to a band of the same size but with different intensities. Rigorous proof by future studies are needed. Missing a band in one of a pair samples suggests a deletion. Invesigations along these lines may lead us to find new polymorphic markers.

Because the majority of the endogenous retroviral sequences in each chromosome do not show polymorphism, we suggest that the banding patterns of

these endogenous retroviruses will be useful for monitoring the human chromosome in human-rodent mono- or subchromosome hybrid cell lines, a technique which may be referred to as LTR band karyotyping. Current conventional methods of monitoring these hybrid cells require skilled labor, and yet the sensitivity is limited. The distribution profiles of HERV-A and RTVL-H2 shown in this paper may be used as references for such purposes. Recently Ledbetter et al. (1990) have described the chromosome-specific patterns of some of the Alu and L1 sequences. This method relies upon PCR, and the resulting patterns are also useful for karyotyping, but the conditions for PCR are critical, and the resulting band intensities can not reflect copy numbers. With our LTR band karyotyping, such problems can be avoided, although use of radioisotopes is necessary. A combination of our and the Ledbetter method may provide a more accurate and efficient way of monitoring chromosomes in hybrid cells.

The physiological roles that these endogenous retroviruses might play are of great interest, although little understood. Tomita et al. (1990) detected the transcripts of HERV-A LTR in a particular lung cancer cell line, and Harada et al. (personal communications.) detected RTVL-H2 transcripts in an embryonic carcinoma cell line. Expression of other endogenous retroviral sequences has also been noted in tumor cells or in placenta. But up to now, intact structure of their transcript have not been reported. Almost all of their sequences have deletion or mutation is its viral genes.

Recently, Heidmann et al. (1991) showed that in place of intact retrovirus transcripts, those having only both LTRs along some short sequences can integrate in genome of EC cell through reversetranscription. The frequency was very low, but this phenomenon suggests that the endogenous retrovirus and its retroposition mechanism may play a great role in insertional mutatageneses. If these insertional mutageneses, occur in early embryonic stage or in germ line, these mutations will be inherited to next generations and will be accumulated in the genome. These accumulations and their influences on neighboring genes may

relate to the change in chromosomes during evolutions process. Detailed analyses of chromosal flanking sequences at the integration site will give further insights into these problems.

## REFERENCES


1. CALLAHAN, R., CHIU, I-M., WONG, J. F. H., TRONICK, S. R., ROE, B. A., AARONSON, S. A., AND SCHLOM, J. (1985). A new class of endogenous human retroviral genomes. *Science* **228**: 1208-1211.

2. CALLEN, D. F. (1986). A mouse hybrid cell psnel for mspping human chromosome 16. *Ann. Genet.* **29**; 235-239.

3. Carlock, L. R., Smith, D., and Wathmuth, J. J. (1986). Geneyic counterselective procdure to isolate interspecific cell hybrids containing single human chromosome: Construction of cell hybrids and recombinant DNA libraries specific for human chromosome 3 and 4. *Somatic Cell Mol. Genet.* **12**: 163-174.

4 DANA, S., AND WASMUTH, J. J. (1982). Linkage of the *leuS, emtB.* and *chr* genes on chromosome 5 in humans and expression of human genes encoding protein synthetic components in human-Chinese hamster hybrids. *Somatic Cell Genet.* **8**: 245-264.

5. DEEN, K. C., AND SWEET, R. W. (1986). Murine Mammary tumor virus pol-related sequences in human DNA: characterization and sequence comparison with the complete murine mammary tumor virus pol gene. *J. Virol.* **57**: 422-432.

6. EMI, M., HORII, A., TOMITA, N., NISHIDE, T., OGAWA, M., MORI, T., AND MATSUBARA, K. (1988). Overlapping two genes in human DNA: a salivary amylase gene overlaps with a gamma-actin pseudogene that carries an integrated human endogenous retroviral DNA. *Gene* **62**: 229-235.

7. FUKUSHIGE, S., MUROTSU, T., AND MATSUBARA, K. (1986). Chromosomal assignment of human genes for gasthrin, thyrotropin (TSH)-$\beta$ subunit and C-erb-2 by chromosome sorting combined with velocity sedimentation and southern hybridization. *Biochemical and Biophysical Research Communications* **134**: 477-483.

8. Gray, J. W., Dean, P. N., Fuscoe, J. C., Trask, B. J., Vanden, G. J., and VanDilla, M. A. (1987). High speed chromosome sorting. *Science* **238**: 323-329.

9. HEIDMANN, O AND HEIDMANN, T. (1991). Retrotransposition of mouse IAP sequence tagged with an indicator gene. *Cell* **64**: 159-170.

10. HARADA, F., AND KATO, N. (1987). Isolation of three kinds of human endogenous retrovirus-like sequences using tRNA Pro as a probe. *Nucleic Acids Res.* **14**: 9153-9162.

11. HAYES, H., KANEDA, Y., UCHIDA, T., AND OKADA, Y. (1987). Regional assignment of the gene for diphtheria toxin sensitivity using subchromosomal fragments in microcell hybrids. *Chromosoma* **96**: 26-32.

12. KAO, F.-T., JONES, C., AND PUCK, T. T. (1976). Genetics of somatic mammalian cells: Genetic, immunologic, and biochemical analysis with Chinese hamster cell hybrids containing a selected single human chromosomes. *Proc. Natl. Acad. Sci. USA* **73**: 193-197.

13. KOI, M., SIMIZU, M., MORITA, H., YAMADA, H., AND OSHIMURA, M. (1989). Construction of Mouse A9 Clones containing a single human chromosome tagged with neomycin-resistancegene via microcell fusion. *Jpn. J. Cancer Res.* **80**; 413-418.

14. KORENBERG, J. R., AND PYKOWSKI, M. C. (1988). Human genome organization: Alu, L1s and the molecular structure of metaphase chromosome bands. *Cell* **53**: 391-400.

15. KRÖGER, B., AND HORAK, I. (1987). Isolation of novel human retrovirus-related sequences by hybridization to synthetic oligonucleotides complementary to the tRNA Pro primer=binding site. *J. Virol.* **61**: 2071-2074.

16. LEDBETTER, S. A., GARCIA-HERAS, J., AND LEDBETER, D. H. (1990). "PCR-karyotype" of humanchromosome in somatic cell hybrids. *Genomics* **8**: 614-622.

17. LEIB-MÖSCH, C., BRACK, R., WERNER, T., ERFLE, V., AND HEHLMANN, R. (1986). Isolation of an SSAV-related endogenous sequence from human DNA. *Virology* **155**: 666-667.

18. LUGO, T. G., HANDELIN, B., KILARY, A. M., HOUSEMAN, D. E., AND FOURINER, R. E. K. (1987). Isolation of microcell hybrid clones containing retroviral vector insertion intospecific human chromosomes. *Mol. Cell. Biol.* **7**: 2814-2820.

19. MAEDA, N. (1985). Nucleotide sequence of the haptoglobin and haptoglobin-related gene pair. The haptoglobin-related gene contains a retrovirus-like element. *J. Biol. Chem.* **260**: 6698-6709.

20. MAGER, D. L., AND FREEMAN, J. D. (1987). Human endogenous retroviruslike genome with type C pol sequences and gag sequences related to human T-cell lymphotic viruses. *J. Virol.* **61**: 4060-4066.

21. MANIATIS, T., FRISCH, E. F.., AND SAMBROOK, J. (1982). "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

22. MARGER, D. L., AND HENTHORN, P. S. (1984). Identification of a retrovirus like repetetive element in human DNA. *Proc. Natl. Acad. Sci. USA* **81**: 7571-7514.

23. MAY F. E., AND WESTLEY, B. R. (1986). Structure of a human retroviral sequence related to mouse mammary tumor virus. *J. Virol.* **69**: 743-749.

24. NAKAMURA, N., SUGINO, H., TAKAHARA, K., CHUNLIAN, J., FUKUSHIGE, S., AND MATSUBARA, K. (1991). Endogeneous retroviral LTR sequences as markers for individual human chromosomes. *Cytogenet. and Cell Genet.* **57**: 18-22.

25. NODA, M., KURIHARA, M., AND TAKANO, T. (1982). Retrovirus-related sequences in human DNA: detection and cloning of sequences which hybridize with the long terminal repeat of baboon endogenous virus. *Nucleic Acids Res.* **10**: 2865-2878.

26. O'CONNELL, C., O'BRIEN, S., NASH, W. G., AND COHEN, M. (1984). ERV3, a full-leugth human endogenous provirus: Chromosomal localization and evolutionary relationships. *Virology* **138**: 225-235.

27: ONO, M., YASUNAGA, T., MIYATA, T., AND USHIKUBO, H. Nucleotide sequence of humanendogenous retrovirus genome related to the mouse mammary tumor virus genome. *J. Virol.* **60**: 589-598.

28. PAULSON, K. E., DEKA, N., SCHIMID, C. W., MISRA, R., SCHINDLER, C. W., RUSH, M. G., KADYK, L., AND LEINWAND, L. (1985). A transposon-like element in human DNA. *Nature (London)* **316**: 359-361.

29. PATTERSON, D., JONES, C., MORSE, H., RUMSBY, P., MILLER, Y., AND DAVIS, R. (1983). Structural gene coding for multifunctional protein carrying orotate phosphoribosyltransferase and OMP decarboxyrase activity is located on long arm of human chromosome 3. *Somatic Cell Genet.* **9**: 359-374.

30. REPASKE, R., STEELE, P. E., O'NEILL, R. R., RABSON, A. B., AND MARTIN, M. A. (1985). Nucleotide sequence of a full-leugth human endogenous retroviral segment. *J. Virol.* **54**: 764-772.

31. SILVER, J., RABSON, A., BRYAN, T., WILLEY, R., AND MARTIN, M., A. (1987). Human retroviral sequences on Y chromosome. *Mol. Cell Biol.* **7**: 1559-1562.

32. TAN, Y. H., TISHFIELD, J., AND RUDDLE, F. H. (1973). The linkage of genes for the human interferon-induced antiviral protein and indophenol oxidaseB traits to chromosome G-21. *J. Exp. Med.* **137**: 317-330.

33. TOMITA, N., HORII, A., DOI, S., YOKOUCHI, H., OGAWA, M., MORI, T., AND MATSUBARA, K. (1990). Transcription of human endogeneous retrovirallong terminal repeat (LTR) sequences in a lung cancer cell line. *Biochemical and Biophysical Research Communications* **166**: 1-10.

34. THOMPSON, L. H., BSCHIINSKI, L. L., STARLLINGS, R. L., DOLF, G., WEBER, C. A., WESTERVERT, A., AND SICHILIANO, M. J. (1989). Complementation of

repair gene mutations on the hemizygous chromosome 9 in CHO: A third repair gene on chromosome 19. *Genomics* **5**: 670-679.

35. VANKEUREN, M. L., HART, I. M., KAO, F. T., NEVE. R. L., BRUNS, G. A. P., KURNIT, D. M., AND PATTERSON, D. (1987). Asomatic cell hybrid with a single human chromosome 22 corrects the kefect in the CHO mutant (Ade-I) lacking adenylosuccinase activity. *Cytogenet. Cell Genet.* 44: 142-147.
36. VANTUINER, P., RICH, D. C., SUMMERS, K. M., AND LEDBETTER, D. H. (1987). Regional mapping panel for human chromosome 17: Application to nurofibromaitosis type 1. *Genomics* **1**: 374-381.
37. Weiner, A. M., Prescott, L. D., and Efstratiady, A. (1986). Nonviral retroposona: genes, pseudogenes, and transposable elements generated by the reverse flow of genetic information. *Ann. Rev. Biochem.* **55**: 631-661.
38. WARBURTON, D., GERSEN, S., YU. M.-T., JACKSON, C., HANDELIN, B., AND HOUSMAN, D. (1990). Momnochromosome human-rodent hybrids from microcell fusion of human lymphoblastoid cells containing an inserted dominant selectable marker. *Genomics* **6**: 358-366.