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# Patterns of Genomic Distribution and Sequence Heterogeneity of a Murine "Retrovirus-Like" Multigene Family

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The mouse genome contains over 100 copies of a dispersed gene family known as "virus-like" genes encoding 30S RNA (VL30). Although they do not share nucleotide sequence homology with known retroviruses, these genetic elements are distinguished by several "retrovirus-like" features, notably, the capacity of the 30S RNA transcripts of these genes to be encapsidated by c-type virions and the transmissibility of VL30 information to other cells via pseudovirion infection. Using VL30 DNA units, cloned from the BALB/c mouse embryonic gene library, we have recently shown that VL30 DNA units share basic structural features with retrovirus proviruses. To shed light on the relatedness of VL30 information to endogenous proviruses and possibly other genetic elements, we extended our previous studies concerning genomic distribution patterns of VL30 elements and patterns of sequence heterogeneity among VL30 units. The following observations were made: (i) VL30 units were distributed among different mouse chromosomes; (ii) distribution patterns of VL30 units markedly differed among mouse strains; (iii) there was constancy of VL30 restriction patterns in different tissues; (iv) a high degree of sequence divergence existed among different VL30 units cloned from the same embryo; and (v) VL30 units were heterogenous with respect to the state of DNA methylation. The results are discussed in terms of the possible modes of evolution of this multigene family.

The mouse genome contains multiple copies of DNA sequences that constitutively express 30S RNA. These cytoplasmic polyadenylated RNA transcripts have been designated "retrovirus-associated sequences" (17) or "virus-like 30S sequences" (VL30) (1) on the basis of their capacity to be efficiently packaged into c-type virions that are assembled in murine cells (1, 9, 17). No nucleic acids sequence homology has been so far detected between VL30 sequences and either exogenous or endogenous retroviruses. However, the "retrovirus-like" character of VL30 sequences is suggested by the following criteria. Expression of VL30 RNA can be induced by halogenated pyrimidines (a feature shared with endogenous retroviruses). In pseudovirions recovered from infected mouse cells VL30 RNA is present in a dimer structure, pseudovirion RNA can be reverse-transcribed by an endogenous primer (1, 9, 17), and, moreover, VL30 RNA can be transmitted to other species via pseudovirion infection (16), where VL30 genetic information ultimately integrates into the cell DNA.

We have recently isolated several VL30 DNA units along with their flanking DNA sequences by molecular cloning in phage λ charon 4A. It was found that the 5.2-kilobase DNA units encoding the 30S RNA are interspersed among variable regions of mouse DNA (11) and that each VL30 unit is distinguished by roughly 400-base-pair long terminal repeats (10). These structural features are common to both retroviruses and transposable elements and prompted us to extend our studies concerning the genetic relatedness of the VL30 gene family to endogenous retroviruses and possibly other cellular genetic elements. To shed some light on these questions, and lacking a more direct approach, we resorted to studies of distribution patterns of the VL30 gene family and patterns of sequence divergence and evolutionary conservation of VL30 DNA sequences.

In this paper, we describe the enumeration of VL30 elements, their distribution throughout the mouse chromosome complement, strain-specific differences in distribution patterns, and analysis of sequence heterogeneity among individual VL30 units. The results are discussed in terms of the possible evolution of this multigene family.

## MATERIALS AND METHODS

**Recombinant DNA clones.** DNA fragments containing VL30 were cloned as previously described (11). Briefly, a gene library was constructed from a partial *Eco*RI digest of BALB/c mouse embryo DNA in the

phage λ charon 4A vector. The gene library was screened with a cDNA probe synthesized from 30S RNA rescued onto murine leukemia virus (MuLV) virions grown on BALB/c-derived JLSV9 cells. Positive hybridization signals were plaque purified and verified as being VL30 DNA clones by virtue of detecting 30S RNA transcripts in RNA blots obtained from uninfected mouse cells.

Heteroduplex analysis was used to select clones that contained complete VL30 units, that is, clones that possessed flanking DNA sequences at both sides of VL30 DNA (11).

Mouse DNA fragments containing VL30 DNA were transferred onto plasmid pBR322 by codigestion with *Eco*RI of equimolar amounts of pBR322 and the respective clone of DNA in λ phage followed by ligation and transformation of *Escherichia coli* HB101 cells. Plasmids harboring VL30 DNA inserts were identified by colony hybridization with a VL30 cDNA probe. In the case of *Eco*RI sites within VL30 DNA, each *Eco*RI fragment was individually cloned in a plasmid.

A cDNA clone of MuLV (7) was obtained from D. Baltimore, and a clone of intracisternal A-particle DNA in plasmid pBR322 (14) was obtained from E. Kuff.

**DNAs of mouse strains and mouse-hamster hybrid cell lines.** High-molecular-weight DNAs from the indicated laboratory mouse strains and from the SC-1 tissue culture line derived from feral specimen of *Mus musculus* were provided to us by E. Kuff and were obtained as described by Kuff et al. (13).

Mouse-hamster hybrid cell DNAs were a gift from R. Perry and P. D'Eustachio. The mouse chromosome complements were described by D'Eustachio et al. (5).

**Agarose gel electrophoresis and restriction enzyme mapping.** DNA fragments were resolved by electrophoresis through 0.6 to 1.0% agarose slab gels and visualized by ethidium bromide staining. Mapping of restriction enzyme recognition sites on cloned DNAs was carried out by use of different combinations of restriction enzymes and size analysis of resultant fragments.

**Hybridization and autoradiography. (i) Blot hybridization.** Electrophoretically resolved DNA fragments were blotted from a gel onto a nitrocellulose filter paper by the Southern procedure (19). Baked nitrocellulose filters were prehybridized for at least 4 h at 65°C in 1× SSC 0.15) M NaCl plus 0.015 M sodium citrate) containing 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, and 50 μg of heat-denatured sonicated salmon sperm DNA per ml. Hybridization was for 18 to 24 h at 65°C in the same solution with the addition of 0.5% sodium dodecyl sulfate and $1 \times 10^6$ to $2 \times 10^6$ cpm of the indicated hybridization probe per ml. Labeling of cloned DNA fragments by nick translation was carried out by the procedure of Roop et al. (15) with one dNTP (600 Ci/mmol) as the labeled precursor. Specific activities of $1 \times 10^8$ to $2 \times 10^8$ cpm per μg of DNA were usually obtained. After hybridization, filters were washed for 2 h at 50°C in 0.1× SSC containing 0.1% sodium dodecyl sulfate, rinsed several times with 0.1× SSC, and air dried. Autoradiography was carried out at −70°C with Agfa Curix RP2 X-ray films and by the use of an enhancing screen.

**(ii) Hybridization kinetics in liquid.** High-molecular-weight DNA was prepared from BALB/c mouse embryos. DNA was sheared by sonication into 4 to 6S fragments. Hybridization mixtures were assembled which contained (per 50 μl) 600 μg of DNA, 3,000 to 8,000 cpm of the indicated cloned DNA fragment that has been labeled with $^{32}P$ by nick translation, 6× SSC, and 0.1% sodium dodecyl sulfate. Samples of 50 μl were sealed in capillaries, incubated for 5 min in boiling water, quenched on ice, and incubated at 65°C. Samples were withdrawn at different times and stored at −70°C. The extent of hybridization was monitored by measuring trichloroacetic acid-precipitable counts after S1 nuclease treatment.

## RESULTS

DNA fragments containing VL30 elements from BALB/c mouse embryos were cloned in bacteriophage λ charon 4A (see above). Restriction enzyme maps were obtained for several VL30 clones, and the location of the long terminal repeats with respect to the insert vector junctions was determined by heteroduplex analysis (11). This enabled us to assign the location of restriction fragments relative to the termini of the VL30 unit. Restriction fragments derived from defined regions of VL30 were subcloned in a plasmid vector. In particular, DNA sequences that reside internally (and are, therefore, devoid of flanking cellular sequences) were cloned. Such "VL30-specific" sequences served as hybridization probes in the experiments described below.

**Enumeration of VL30 units.** When the mouse gene library (in a phage vector) was challenged with VL30-specific DNA probes (cloned in a plasmid vector), VL30-containing DNA fragments were encountered at a frequency suggesting that VL30 elements are reiterated 100 to 200 times in the mouse genome. This estimate, however, might be inaccurate due to a biased sequence representation in the gene library. Therefore, we obtained an independent measure of VL30 copy number by following the annealing kinetics between $^{32}P$-labeled, cloned VL30 sequences and excess DNA from uninfected mouse embryos (Fig. 1). The hybridization kinetics was measured relative to those of two other endogenous retrovirus-related gene families, namely, MuLV-related endogenous proviruses and intracisternal A-particle genes. The reassociation kinetics of the slow-reassociating fraction of mouse DNA was also monitored to serve as a standard for "unique" sequences. From the $C_0t_{1/2}$ values obtained, we estimated that approximately 150 VL30 copies are present in the mouse genome. This estimate is somewhat larger than previous estimates based on hybridization studies with the 30S RNA transcripts of these genes (1, 9). Very similar hybridization kinetics were obtained with two different VL30-specific probes (Fig. 1), thereby reducing the possibility of overestimation due to the presence

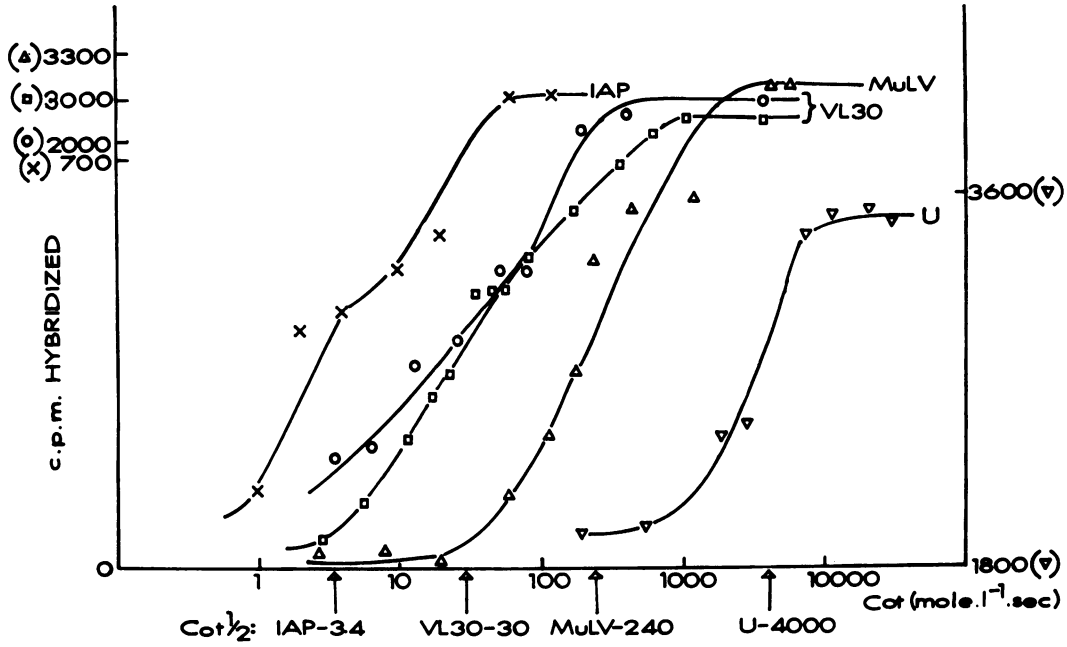


FIG. 1. Kinetics of hybridization between cloned VL30 DNA sequences and mouse DNA. Annealing reactions between the indicated $^{32}P$-labeled cloned DNA fragments and excess DNA from uninfected BALB/c mouse embryos were performed as described in the text. The following cloned DNA fragments (all in plasmid PBR322) were used: (○, □) 0.9- and 1.1-kilobase adjacent VL30 DNA fragments located internally in respect to the long terminal repeat; (×) intracisternal A-particle (IAP) sequences; (△) cDNA clone of MuLV. Also shown is the reassociation kinetics of the slow-reassociating fraction of mouse DNA that has been uniformly labeled with $^{32}P$ and annealed under the same conditions (▽). The different extents of hybridization achieved are, at least in part, due to different insert-vector length ratios in the different plasmid probes. Therefore, to better illustrate relative kinetics of hybridizations the counts per minute hybridized are presented in arbitrary scales. The $C_0t$ values presented are corrected values for standard salt conditions (2).

of extra non-VL30 cellular sequences within the cloned VL30 copy used as a probe (see below).

The genomic reiteration frequencies calculated for endogenous MuLV-related sequences (calculated 17 per genome) and intracisternal A-particle genes (calculated, 1,200 per genome) are in good agreement with previous estimates (14, 20). This result places the VL30 gene family in an intermediate position with respect to its reiteration frequency relative to the two other families of retrovirus-related genes of *M. musculus* examined in this experiment.

**VL30 genes distributed among different mouse chromosomes.** Our previous studies have shown that the 5.2-kilobase DNA units encoding 30S RNA are interspersed among variable flanking regions of mouse DNA (11). The methods employed in the previous studies, however, could not resolve whether VL30 DNA elements are all clustered on one chromosome or are alternatively distributed at different chromosomes. To answer this question, we used a panel of mouse-hamster hybrid cell lines that possess defined sets of different mouse chromosomes. DNA digests obtained from several such mouse-hamster hybrid cell lines were electrophoresed, blotted, and hybridized with VL30-specific probes (Fig. 2).

The analysis of hybridizing bands is simplified due to the fact that VL30 sequences are grossly species specific. This is evident by the lack of any hybridization with hamster DNA. Therefore, all of the hybridization bands detected in mouse hamster-hybrid cell lines are attributable to the presence of these sequences on mouse chromosomes. It is clear that in mouse-hamster cell lines containing different complements of mouse chromosomes different subsets of VL30 bands are detected, indicating that VL30 copies are distributed among different mouse chromosomes. A more thorough analysis is required, however, to assign individual hybridization bands to specific chromosomes. Moreover, the data shown do not rule out the possibility that most VL30 genes are clustered on a particular chromosome.

**Differences in genomic restriction patterns in different mouse strains.** High-molecular-weight

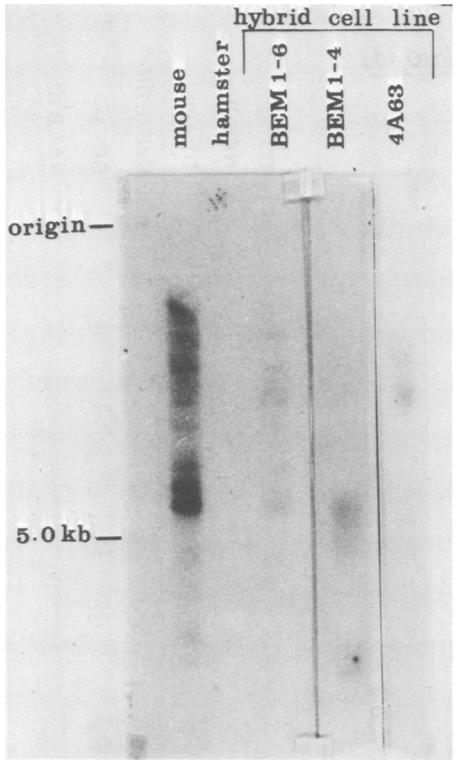


FIG. 2. Blot analysis of VL30 genes in *Eco*RI-digested DNA from mouse, hamster, and mouse-hamster hybrid cells. *Eco*RI-digested DNA from mouse, hamster, and three mouse-hamster hybrid cells were electrophoresed through an agarose gel, blotted. and hybridized with a mixture of the two VL30-specific probes described in the legend to Fig. 1. Hybridization conditions were as described in the text. The chromosome complements of the hybrid cell lines used were as described by D'Eustachio et al. (5).

DNAs from different inbred strains of *M. musculus* and DNA derived from one feral specimen of *M. musculus* were digested with different site-specific endonucleases and analyzed by blot hybridization for the distribution of VL30-containing DNA fragments (Fig. 3). There are certain difficulties in performing this type of analysis with a multigene family as reiterated as the VL30 family, namely, the large number of hybridization bands and the resultant masking of differences in restriction patterns due to poorly resolved multiple bands. To reduce these factors, restriction enzymes were chosen which also cut within VL30 DNA twice or more (*Xba*I and *Hin*dIII in Fig. 3). With these enzymes, the bulk of the hybridization is cleared onto a few bands of a smaller size and a high-intensity signal, whereas the larger fragments, mostly VL30 cell-junction fragments, are better resolved. This procedure disclosed marked differences in VL30 distribution patterns for each mouse strain, yet a substantial number of bands appeared to be shared by DNA of most of the strains analyzed.

**Constancy of VL30 restriction patterns in different tissues.** High-molecular-weight DNAs from three differentiated tissues of a BALB/c mouse (liver, spleen, and kidney), DNA from germ-line cells, and DNA prepared from a whole BALB/c mouse embryo were digested with *Eco*RI and analyzed by blot hybridization for the distribution of VL30-containing DNA fragments (Fig. 4). As shown in Fig. 4, the band patterns of DNAs from all five sources were indistinguishable from one another. We also failed to detect tissue-specific differences with DNA digests obtained by other restriction enzymes like *Xba*I and *Hin*dIII (data not shown). Since most of the detected bands were composites of viral and cellular sequences, we can conclude that there are no gross rearrangements of VL30 DNA upon tissue differentiation.

**Pattern of sequence heterogeneity among individual VL30 units.** Comparative restriction mapping studies are a sensitive tool for detecting scattered base differences among closely related sequences. The basis of this approach is that even a single base substitution might create or destroy a restriction site. To assess the degree of sequence divergence among VL30 units of the same individual, VL30 genomic clones were mapped with several restriction enzymes that recognize a six-base sequence. Comparative maps of two randomly cloned VL30 units are shown in Fig. 5. The two maps are very different. Only 3 of 10 or 12 recognition sites mapped are shared by both clones, indicating a high degree of sequence divergence. However, the comparison of two clones arbitrarily chosen from the repertoire of 100 to 200 VL30 units might be misleading. To obtain a more quantitative measure to the degree of sequence divergence of VL30 genes, the following experiment was carried out. DNA from a BALB/c mouse embryo was digested with *Hin*dIII, an enzyme shown in preliminary experiments to produce three internal cuts in many cloned VL30 DNAs. DNA fragments were resolved by electrophoresis, blotted, and hybridized with a $^{32}$P-labeled subclone of VL30 that contained less than 1 kilobase of VL30 sequences located roughly at the center of the element (Fig. 6). The rationale of this experiment is that this probe will anneal with a limited VL30 sequence confined within one or two internal fragments. Therefore, a simple band pattern is expected from total genomic digest. For example, if all 100 to 200 VL30 copies were identical only one or two hybridization bands would be detected, whereas sequence alterations in internal *Hin*dIII recogni-

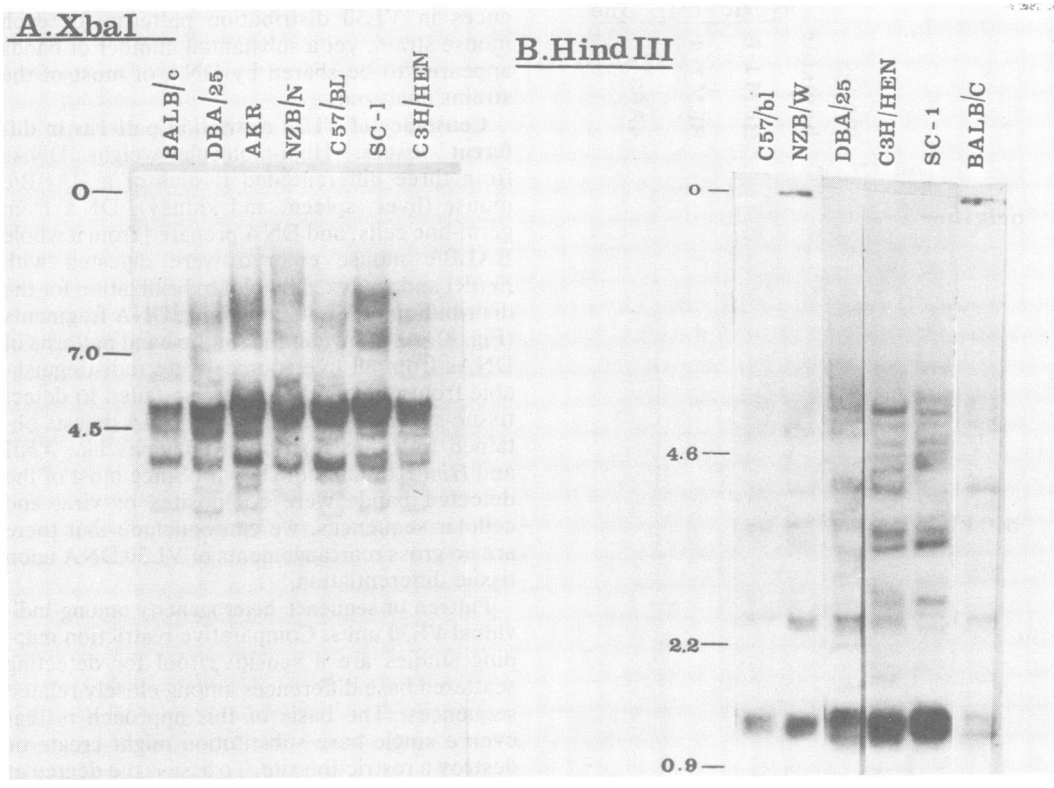


FIG. 3. Restriction enzyme patterns of cellular DNAs from different mouse strains. Fifteen micrograms of DNA from each indicated mouse strain was digested to completion with the indicated enzyme, electrophoresed through a 0.7% agarose gel, transferred to a nitrocellulose membrane filter, hybridized, and autoradiographed. The hybridization probe was a mixture of cloned VL30 DNA fragments that collectively cover 2.7 kilobases of internally located VL30 DNA sequence.

tion sites in some VL30 units would produce additional bands. As shown in Fig. 6, after a short autoradiographic exposure, the hybridization was indeed observed, mostly in two closely migrating *Hin*dIII fragments. In longer exposures, however, many larger additional bands were detected. These bands are likely the result of nucleotide substitutions within the nucleotide sequences comprising internal *Hin*dIII sites in some VL30 units. The ratio of hybridization in the additional bands relative to the amount of hybridization in the two major bands (determined by autoradiogram scanning) is, therefore, a direct measure of sequence heterogeneity at these particular sites. From the autoradiogram scan shown in Fig. 6, we conclude that approximately one-third of VL30 units in BALB/c mouse embryo DNA have undergone sequence substitutions at these particular sites.

In a parallel experiment where a probe derived from a different region of VL30 DNA was used similar results were obtained (data not shown). This reduces the possibility of overestimation due to the fact that the probe used in the experiment shown in Fig. 6 was derived from a hypervariable region within VL30 DNA. The detection of the larger hybridizing *Hin*dIII fragments could also be the result of insertion of cellular sequences within VL30 copies. Further experiments are required to distinguish between these two possibilities.

**Heterogeneity in methylation pattern of VL30 genes.** It has been recently shown that the level of expression of certain endogenous proviruses correlates with the level of DNA methylation (8). With the notion that probably only a fraction of VL30 units are transcriptionally active, we examined the state of VL30 DNA methylation in mouse embryonic DNA. *Hpa*II and *Msp*I isoschizomers were used to differentiate methylated from nonmethylated DNA sequences (22). Hybridization patterns that are too complex are anticipated from digests with these enzymes that recognize a four-base sequence, considering the genetic complexity of VL30 DNA, the multiplicity of VL30 units, and their sequence heteroge-

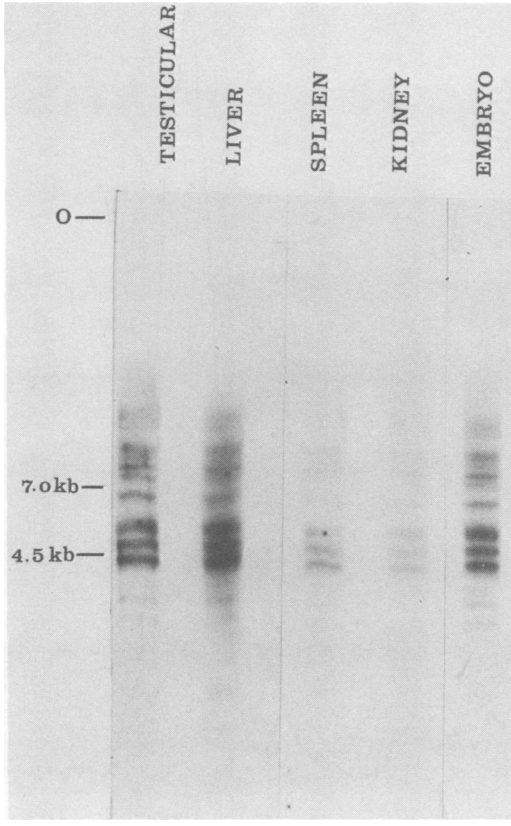


FIG. 4. Restriction enzyme patterns of cellular DNA from various tissues of a BALB/c mouse. Fifteen micrograms of DNA from each tissue was digested with *Eco*RI, electrophoresed, and hybridized as described in the legend to Fig. 3.

neity. To obtain simpler band patterns, we used a similar approach exploited in the experiment described above, namely, blot hybridization with a probe that detects only a short, centrally located VL30 sequence spanning very few *Hpa*II or *Msp*I sites (none of which produce VL30-cell junction fragments) (Fig. 7). *Msp*I, which cleaves CCGG sequences regardless of their methylation status, produced two predominant bands from the total genomic digest (the minor larger fragments are likely a reflection of VL30 sequence heterogeneity). *Hpa*II, which cleaves only nonmethylated sequences, also produced the same two bands. In contrast, however, the bulk of VL30 sequences were contained in relatively large fragments. It appears, therefore, that VL30 units can be divided into two subpopulations in respect to the methylation status of these particular sites in their DNA. In the *Hpa*II digest the majority of the hybridization was found in fragments whose size exceeded the VL30 unit size (5.2 kilobases), indicating that although the probe covered only a short DNA stretch, the remainder of *Hpa*II sites in this fraction of VL30 copies were also methylated. This result is consistent with the notion that only a fraction of VL30 elements are transcriptionally active.

## DISCUSSION

The genetic relatedness of VL30 DNA to retroviruses is not clear. The retrovirus-like character of VL30 DNA is suggested by a structural organization that is basically similar to that of retroviral proviruses and by several functional parallels with retroviruses such as efficient RNA encapsidation, transmissibility of RNA via pseudovirion infection, and subsequent reverse transcription with an endogenous primer. These features are interpretable either as the consequences of degeneration process of once-competent endogenous proviruses or às representing intermediates in the evolution of retroviruses from cellular genetic information (21). A major difference from other families of endogenous retroviruses is that a putative nondefective progenitor of these genetic elements has not been identified. In this respect VL30 genes closely resemble intracisternal A-particles that also share common structural features with transmissible retroviruses and yet are not known to have an infectious extracellular form (13).

A-particle sequences have been found in a transmissible virus, M432, derived from the species *Mus cervicolor* (12). This virus, however, contains only a limited subset of the A-particle sequence. These sequences are thought to have been acquired through recombination with A-particle genes endogenous to *M. cervicolor* (R. Callahan, K. K. Lueders, E. L. Kuff, and E. Birkenmeir, personal communication).

Despite this fact, the possibility that these

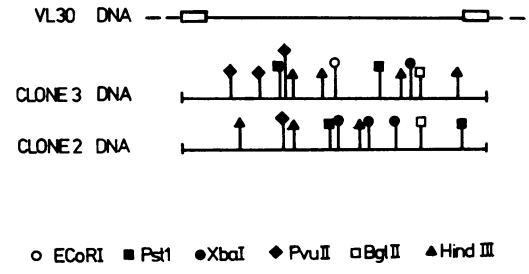


FIG. 5. Restriction enzyme maps of two VL30 DNA clones. Recognition sites of six different restriction enzymes on two VL30 DNA clones were mapped. Location of the long terminal repeats (▭) and junctions of VL30 DNA and flanking sequences (— —) were determined by heteroduplex analysis (11) to allow alignment of the two maps.

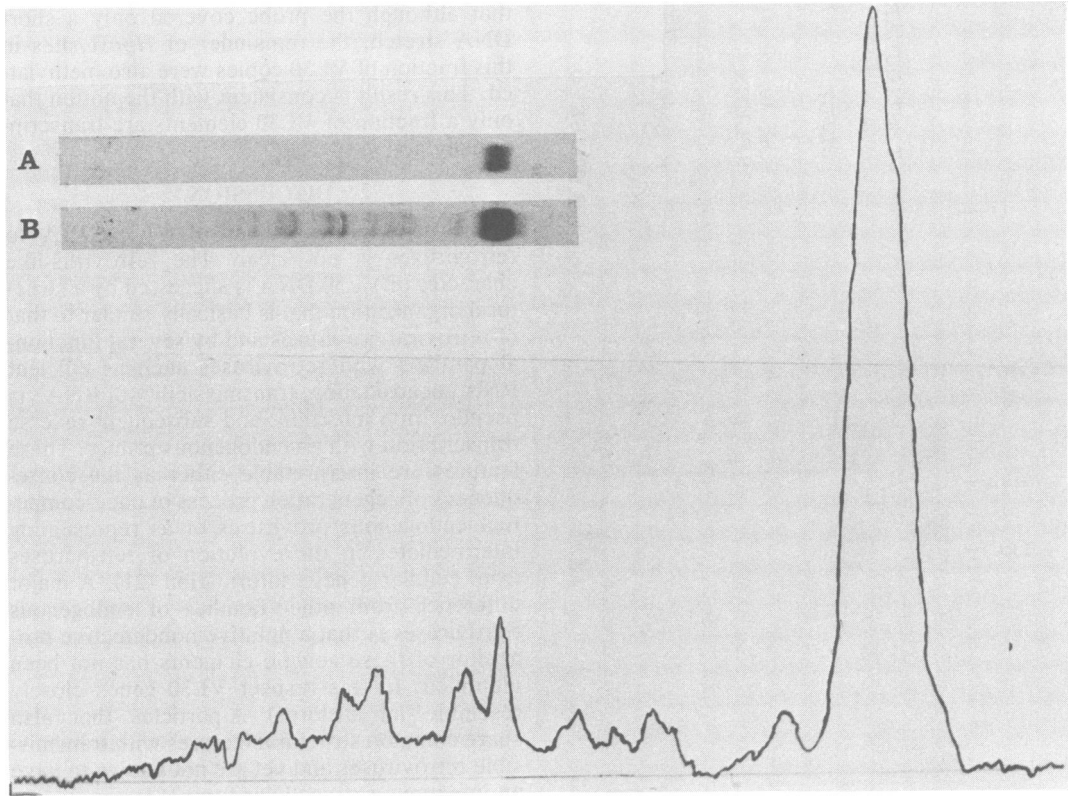


FIG. 6. Evaluation of degree of heterogeneity in restriction enzyme sites among VL30 units. Fifteen micrograms of DNA from a BALB/c mouse embryo was digested with *Hin*dIII and blot hybridized with the nick-translated 0.75-kilobase VL30 DNA fragment (the *Eco*RI-*Pst*I fragment located to the right of the central *Eco*RI site of clone 3 shown in Fig. 5). The autoradiogram was scanned in a Gilford model 2400 spectrophotometer. Inserts A and B are short and longer exposures, respectively, of the autoradiogram whose scan is shown.

components entered the mouse genome through infection of a once-competent retrovirus should be considered when evaluating possible modes of evolution of VL30 genes.

Another feature that distinguishes the VL30 gene family is its genomic reiteration. VL30 units are 1 order of magnitude more abundant in *M. musculus* than are MuLV-related endogenous proviruses or murine mammary tumor virus (MMTV)-related endogenous proviruses. Therefore, any proposed scheme for the evolution of VL30 genes should account for these two points, namely, the multitude of VL30 units and the incompetence of all contemporary VL30 copies, in particular when considering the relative significance of reoccurring independent germ-like infections on one hand and the evolution within the cell through amplification of a limited number of elements (either ancestral proviruses or cellular genes) on the other hand.

Different mouse strains exhibit marked differences in the pattern of VL30-containing genomic fragments (Fig. 3). Similar strain-specific distribution patterns were reported for other endogenous retrovirus-related gene families of *M. musculus*, MMTV-related endogenous proviruses (4), and MuLV-related endogenous proviruses (3). The emergence of differences in restriction patterns of MMTV proviruses among strains has been interpreted as the consequence of recent and even ongoing entries of proviral sequences into germinal cells by independent infections (4). On the other hand, it was suggested that instability of proviral DNA might be responsible for the observed differences, specifically for repositioning within the cell of established proviruses (3). From the consideration mentioned above, it seems likely that the marked differences in distribution patterns of VL30 sequences that emerged on such a short time scale of inbreeding (50 to 60 years) are, at least in part, the result of dynamic rearrangements of already established VL30 elements (whatever their origin). This may include recombination with other genetic elements, repositioning (either via RNA intermediate or DNA-DNA transposition), and

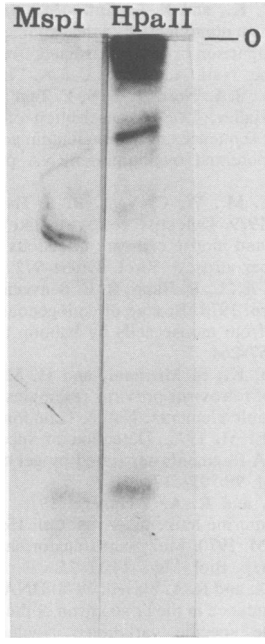


FIG. 7. Blot hybridization analysis of VL30-containing DNA fragments in *Hpa*II- and *Msp*I-digested mouse DNA. BALB/c mouse DNA was digested with either *Msp*I or *Hpa*II. Hybridization was with the same VL30 probe used in the experiment shown in Fig. 6.

amplification. Studies of these putative processes are hampered by the multitude of VL30 genes and the resultant complexity of restriction patterns. Study of cells in which a limited number of VL30 copies were introduced into background-free DNA of a heterologous cell (16) might facilitate this analysis.

Comparison among individual VL30 units cloned from the same embryo shows considerable sequence divergence (Fig. 5 and 6). This apparent high mutation rate of VL30 DNA might be related to the fundamental question of whether VL30 sequences have some general role in cell physiology. According to the neutral mutation hypothesis, the rate of nucleotide substitutions is expected to be higher for functionally less important genes. In the case of a multigene family such as VL30, the large reservoir of genes would allow a high level of mutation even if VL30 genes were functionally important. It is likely, therefore, that only a fraction of the elements will remain transcriptionally active. The methylation patterns presented here are indeed consistent with the notion that only a fraction of VL30 genes are transcriptionally active. However, this question should be approached through detailed structural analysis. Recombination of VL30 with other cellular sequences may also contribute to the apparent sequence heterogeneity. It has been demonstrated that in cases where the generation of transforming virus is brought about through acquisition of rat sequences, recombination with rat 30S sequences occurs concomitantly with the acquisition of the cellular oncogene (6). It is reasonable to assume that these recombination events represent a private case for the recombination of 30S sequences with sets of cellular sequences, detected by virtue of the strong selection for cellular transformation. Such cellular acquisitions might be the reason for the detection of "bubbles" of nonhomologous sequences that we have previously encountered by heteroduplexing certain pairs of VL30 DNA clones (11). Sequential acquisition and subsequent modification of sets of cellular genes has been proposed as the mode of evolution of retroviruses from cellular elements (18).

So far, proteins encoded by VL30 sequences have not been identified. It is thus not clear whether VL30 sequences play a role in the cell. Nevertheless, when considering selective pressures that may operate on the system, possible indirect effects of VL30 genes on the cell phenotype should be taken into account. We have observed that VL30 long terminal repeats possess a promotor activity (unpublished data). Therefore, VL30 units may effect the expression of neighboring cellular genes. The significance of these effects, if any, depends on both promotor strength and participation of VL30 genes in DNA rearrangements that may lead to creation of novel genomic linkages. In this regard, the abundance of VL30 element, relative to other endogenous retrovirus-related families, might be an important factor.

## ACKNOWLEDGMENTS

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