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Discrete regions of sequence homology between cloned rodent VL30 genetic elements and AKV-related MuLV provirus genomes

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ABSTRACT

Southern blot analyses using reduced stringency hybridization conditions have been employed to search for sequence homologies between rodent VL30 genes and murine leukemia virus (MuLV) proviruses. These constitute two classes of transposon-like elements previously believed to be genetically unrelated. Our results demonstrate that cloned representatives of both ecotropic and xenotropic-like proviruses share discrete regions of sequence homology with VL30 genes of both rat and mouse origin. These regions of homology exist in both 3' and 5' halves of the MuLV genome but do not include extensive portions of the long terminal repeat (LTR) or a 0.4 Kbp segment of the *env* gene specific for recently acquired ecotropic-type MuLV proviruses. DNA sequencing, however, revealed that the short inverted terminal repeat sequence of MuLV proviral LTRs is almost perfectly conserved at the terminus of an integrated mouse VL30 gene. These results suggest that recombination events with rodent VL30-type sequences occurred during early MuLV evolution. The strong conservation of the inverted terminal repeat sequence may reflect a common integration mechanism for VL30 elements and MuLV proviruses.

INTRODUCTION

Mouse VL30 genes are a dispersed class of moderately repetitive sequence elements with structural features generally analogous to integrated forms of retrovirus proviruses and certain classes of transposable genetic elements (1-4). They are 5-6 Kbp long and are flanked by 0.4 to 0.5 Kbp long terminal repeat (LTR) sequences (5). Recent studies from our laboratory have shown that VL30 elements are present in the genomes of all mice examined, irrespective of species or geographic origin, and are genetically related to a similar class of sequences in the rat genome (6). Furthermore, the arrangement of VL30 sequences in the genome is polymorphic in different mice and at least one of these polymorphisms reflects the differing chromosomal location of a complete VL30 sequence element (6). VL30 sequences, therefore, appear to have arisen in early rodent ancestors and have been mobile at some point during their evolution.

The predominant transcription product of VL30 genes is a 30S polyadeny-

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lated RNA (1,7,8). This RNA can be copackaged into the virions of either infecting or activated endogenous murine leukemia viruses (MuLV) and transmitted from cell to cell (8,9). Transmission via mixed pseudotype virions is, therefore, a major candidate to explain VL30 gene mobility. However, VL30 genes have been reported to contain little or no sequence homology to MuLVs of either ecotropic or xenotropic host range specificity (1,7,8). It has, therefore, not been clear how copackaging with MuLV genomic RNA might be facilitated.

In the present study, we have reexamined the issue of sequence homology between cloned VL30 genes from rodents and MuLV proviral DNA by blot hybridization utilizing moderately stringent conditions. The results suggest a complex evolutionary relationship, possibly involving recombination events, between MuLV proviruses and rodent VL30 genes. Such recombination events may represent naturally occurring analogues to the interactions between MuLV, rat VL30 and other cellular sequences which led to the derivation of Harvey and Kirsten strains of murine sarcoma viruses (10-14).

MATERIALS AND METHODS

Clones

Clone 27A, as a 5.5 Kbp *Sac* I insert in λ.gtWes.λB, representing a member of an endogenous rat VL30 gene family (15,16) was obtained from Dr. H.A. Young, BRL, Inc., Bethesda, MD. Mouse VL30 clone, BVL-1, was isolated in our laboratory from a Balb/c mouse genomic library in λ Charon 4A phage (2). BVL-1 was derived as a 13 Kbp *Eco*RI insert containing a 5.2 Kbp VL30 sequence flanked by about 7 and 0.3 Kbp of unrelated mouse DNA. Clone pHR7, a subclone of BVL-1 in pBR322 contains a 1.6 Kbp *Eco*RI/*Hin*d III fragment which includes one complete copy of the VL30 LTR. AKR murine leukemia viral DNA (AKV) as a cloned representative of an ecotropic retrovirus was derived in a permuted form as an 8.3 Kbp insert in pACYC177 at the unique *Hin*d III site. This clone, designated pAKV177, was constructed from a corresponding clone of AKV in λ phage Charon 21A. Dr. D. Steffen, Worcester Foundation for Experimental Biology, Boston, MA, provided us with the original λ AKV clone and AKV-related endogenous, presumably xenotropic, proviral clones, 14-9 and 36.1 (5' and 3' MuLV-related sequences, respectively), which were originally isolated by Roblin et al. from a C3H/HeN mouse genomic library (17).

Restriction Endonucleases

Restriction endonucleases were obtained from New England BioLabs,

Boehringer Mannheim Corp. and Bethesda Research Laboratories. Generally, the digestion conditions recommended by the supplier were used.

Hybridizations

Southern blot hybridizations were performed by the method of Jeffreys and Flavell (18), except that the gels were first treated with 0.25 M HCl solution for 40 min to facilitate the transfer of high molecular weight DNA fragments (19). The blots were then hybridized to [^{32}P]-nick translated (20) probes in 6 X SSC, 1X Denhardt's solution, 50 µg/ml Herring sperm DNA, 10 µg/ml poly(A), and 0.1% SDS, at 55° C for about 2 days. Dot hybridizations were performed as described by Kafatose et al. (21) using the BRL hybri-dot manifold (BRL, Inc., Bethesda, MD).

Thermal Denaturation

Following post-hybridization washes the dots or bands on the filter containing the respective DNAs hybridized to ^{32}P-labeled, pAKV177 probe, were excised and placed in liquid scintillation vials. The percent of total filter-bound ^{32}P-radioactivity solubilized in 0.1 X SSC or 1.0 X SSC as a function of 5° C-increment in temperatures (Thermocirculator, Beckman, Inc.) was determined by liquid scintillation counting.

DNA Sequencing

DNA sequencing was done essentially according to the method of Maxam and Gilbert (22), using the VL30 LTR-containing subclone pHR7.

RESULTS

Regions of Rat and Mouse VL30 Genes Homologous to AKV DNA

Restriction endonuclease digests of rat 27A and mouse BVL-1 VL30 DNAs were electrophoresed, blotted, and then hydridized with [^{32}P]-labeled AKV DNA under moderately stringent conditions (6 X SSC/55° C). Figure 1 illustrates a representative Southern blot analyses of rat 27A DNA (lanes 1 through 6) and mouse BVL-1 DNA (lanes 7 through 12). Some, but not all restriction fragments derived from the respective VL30 sequences were observed to cross-hybridize with the AKV probe. To examine the relative stability of the hybrids, post-hybridization washes of the blots were performed under increasingly stringent conditions (Aa, 6 X SSC/55°; Ab, 3 X SSC/65° C; and Ac, 0.1 X SSC/65° C). It is interesting to note that AKV hybrids with mouse BVL-1 restriction fragments were highly unstable compared to the corresponding hybrids with rat 27A fragments (Fig. 1 Ab and Ac).

The restriction enzyme fragments derived from either rat 27A (Fig. 2A) or mouse BVL-1 (Fig. 2B) clones, which hybridized with the AKV probe, are shown in figure 2 as open bars aligned below the respective physical maps

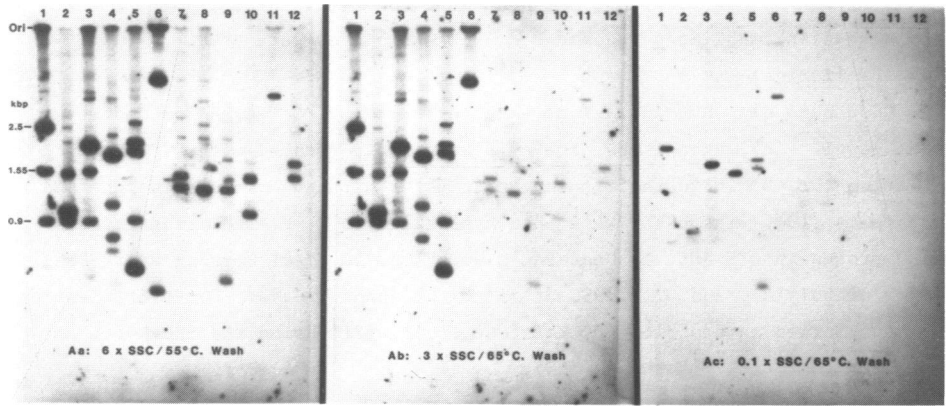

Figure 1. Southern blot analysis of rat (clone 27A) and mouse (clone BVL-1) VL30 DNAs. Restriction digests of cloned rat 27A (5.5 Kbp *Sac* I insert/lanes 1 through 6) and mouse BVL-1 (lanes 7 through 12) DNAs were electrophoresed in a 1.4% agarose gel, blotted to nitrocellulose membrane filters and then hybridized to ^{32}P-nick translated AKV probe as described in the Methods. Post-hybridization washes were performed under increasingly stringent conditions: Aa) 6 X SSC at 55° C; Ab) 3 X SSC at 65° C; and Ac) 0.1 X SSC at 65° C. Lanes 1) *Eco*RI; 2) *Eco*RI/*Hin*d III; 3) *Eco*RI/*Pst* I; 4) *Eco*RI/*Bgl* II; 5) *Hin*d III/*Pst* I; 6) *Kpn* I; 7) *Hin*d III; 8) *Hin*d III/*Xho* I; 9) *Hin*d III/*Kpn* I; 10) *Hin*d III/*Pst* I; 11) *Bam* HI; and 12) *Bam* HI/*Pst* I.

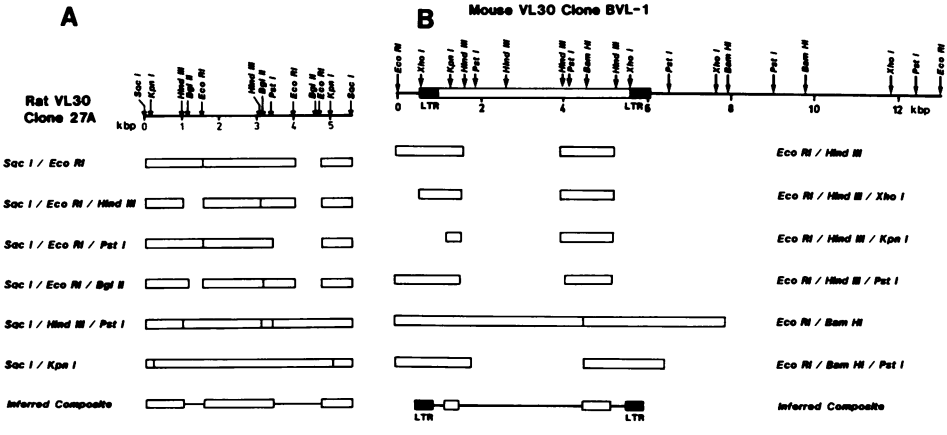

Figure 2. Regions of rat 27A and mouse BVL-1 DNAs homologous to AKV. Restriction endonuclease maps of rat 27A (A) and mouse BVL-1 (B) VL30 clones relative to their *Sac* I and *Eco*RI termini, respectively: The opened bars represent corresponding restriction fragments of 27A or BVL-1 DNA's hybridized to [^{32}P]-nick translated AKV DNA. Inferred composite indicates maximal rather than absolute, regions of either 27A or BVL-1 DNAs homologous to AKV genome.

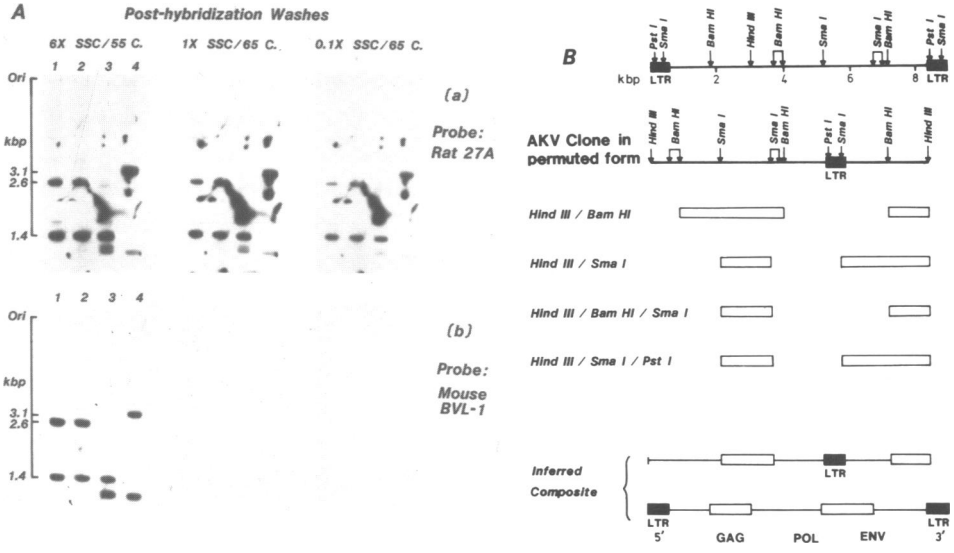

Figure 3. Regions of AKV genome homologous to both rat (27A) and mouse (BVL-1) VL30 clones. (A) Southern blot analysis: Restriction digests of an isolated 8.3 Kbp AKV insert (cloned at *Hin*d III site in pACYC177) were electrophoresed in a 1% agarose gel, blotted, and hybridized to [^{32}P]-nick translated rat 27A clone (a) or mouse VL30-specific DNA fragments internal to VL30 gene after a *Hin*d III/*Xho* I digestion of clone BVL-1 (b), as described in the Methods. Lanes 1) *Sma* I; 2) *Sma* I/*Pst* I; 3) *Bam* HI/*Sma* I; and 4) *Bam* HI. (B) Restriction endonuclease maps of AKV and of its permuted form relative to *Hin*d III termini. The opened bars represent the corresponding restriction fragments of AKV DNA hybridized to both rat and mouse VL30 probes. The approximate locations of *gag*, *pol*, and *env* genes in the non-permuted form of AKV are shown at the bottom of the figure. Inferred composite indicates maximal, rather than absolute, regions of AKV DNA homologous to rat and mouse VL30 clones.

of the VL30 clones. Based on this data, a composite was inferred for each clone which indicates the maximal regions of sequence homology to AKV in different regions of the corresponding VL30 clones. Evidently, there are at least two discrete regions in the mouse VL30 clone, and three in the rat VL30 clone which contain AKV-related sequences. Moreover, these homologous regions did not appear to include LTR termini of the VL30 sequence in mouse clone BVL-1 (Fig. 2B, also see below) as evidenced by a lack of hybridization to several fragments containing LTR sequences. The presence of repeated termini in the *Sac* I-derived rat clone 27A is uncertain (15,16).

Regions of AKV Genome Homologous to Rat and Mouse VL30 Clones

When complementary Southern blot analyses were performed, virtually identical sets of AKV restriction fragments were found to hybridize to rat

and mouse VL30 probes (Fig. 3A). The results, summarized in figure 3B, demonstrate that AKV DNA contains two discrete regions of sequence homology with rodent VL30 elements. One of these regions is encompassed within a 1.2 kbp *Bam* HI/*Hin*d III fragment lying mostly within the *gag* gene region (23), while the other is contained within a 1.4 kbp *Sma* I fragment containing both *pol* and *env* sequences (23). No hybridization was observed to fragments derived from the LTR. Furthermore, consistent with the data described in figure 1, hybrids between AKV restriction fragments and the rat VL30 probe were more stable relative to the mouse VL30 probe on washing the blots under higher stringency conditions. These hybrid stability analyses again suggested that the VL30 related sequences in the AKV genome were more closely related to those of rat clone 27A rather than the mouse VL30 clone BVL-1.

In order to better quantitate the hybrid stability analyses, direct measurements were made of the thermal stabilities of AKV:VL30 hybrids as follows: The respective λ-phage clones AKV, 27A, and BVL-1 were applied as dots to a sheet of nitrocellulose paper, hybridized with a ^{32}P-labeled, gel purified, AKV probe prepared from the plasmid clone pAKV177, and thermally eluted in buffers of varying ionic strength. The results (not shown) demonstrated the following: 1) Rat clone 27A DNA consistently hybridized 5 to 6 fold more radioactivity on a per μg basis than mouse clone BVL-1 DNA; 2) hybrids with rat 27A DNA were moderately but consistently more stable than the corresponding hybrids with mouse BVL-1 DNA; and 3) the reductions in Tm of the various heterologous hybrids were consistent with an average base sequence divergence of 10 to 30 percent between AKV proviral DNA and the two VL30 clones (24,25).

Cross-Species Homology Within VL30 Genes

The findings that both rat and mouse VL30 probes hybridize to the same set of AKV restriction fragments is consistent with previous reports of genetic relatedness between rat and mouse VL30 sequences (6). In order to further study this aspect, the conserved sequences in the two VL30 clones were partially mapped by Southern blot analysis. The results are summarized in figure 4. Each VL30 clone was found to contain blocks of conserved sequences interspersed with regions of apparent non-homology. A comparison of the maps obtained with those of figure 2 indicate that these conserved regions generally correspond with regions containing AKV-related sequences although some differences were noted. In particular, the rightward segment of rat clone 27A contains a region of AKV-related sequences (Fig.

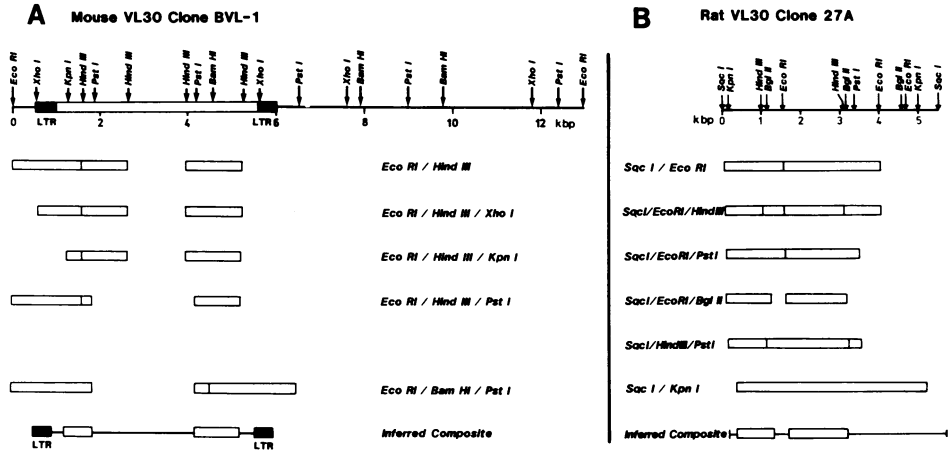

Figure 4. Restriction endonuclease maps of mouse BVL-1 (A) and rat 27A (B) clones. The opened bars represent the corresponding restriction fragments hybridized to 27A clone in the map of mouse BVL-1 clone (A) or to mouse VL30-specific DNA fragments in the map of rat 27A clone (B). Southern blot analyses (not shown) were performed essentially as described in the Methods. Inferred composite indicates maximal possible regions of mouse BVL-1 (A) or rat 27A (B) DNAs homologous to 27A or BVL-1 probes, respectively.

2A) but no sequences related to mouse VL30 (Fig. 4B). This observation is consistent with the increased specific activity of hybridization of AKV to the rat VL30 clone and suggests that more detailed mapping of the VL30-related sequences in AKV might reveal sequences specific for rat VL30.

VL30 and AKV Long Terminal Repeats Do Not Cross-Hybridize

The data presented in figures 1 and 3 indicated that the observed sequence homology between AKV and the cloned mouse VL30 gene did not include the LTR regions. To further substantiate this observation, a *Sma* I/*Pst* I double-restriction enzyme digest of AKV DNA, which generates an approximately 0.5 Kbp fragment containing exclusively LTR sequences (17), was probed with ^{32}P-labeled pHR7 DNA, a subclone of BVL-1 in pBR322, by Southern blot hybridization using reduced stringency conditions. The pHR7 DNA, which contains a 1.6 Kbp *Eco*RI/*Hind* III insert derived from the left end of clone BVL-1 (for restriction enzyme map of BVL-1, see Fig. 2B), was chosen as an LTR probe because of two important reasons: a) since it is highly enriched in LTR sequences it can be nick translated to relatively high specific radioactivity within these sequences, and b) the fact that it also includes a block of non-LTR, but AKV-related sequences, provided a positive internal control to detect both LTR and non-LTR sequence homology

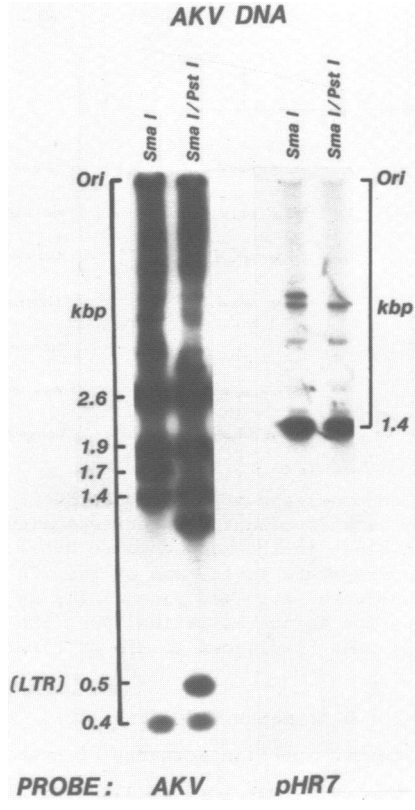

Figure 5. Determination of sequence homology within LTR regions between AKV and mouse VL30 clones by Southern blot hybridization analysis. Restriction enzyme digests of gel-purified, 8.3 Kbp AKV DNA were electrophoresed in two separate runs, blotted, and then hybridized to ^{32}P-labeled AKV or pHR7, DNA probes as indicated in the figure. The expected size of the various restriction enzyme fragments are shown in the figure which includes the 0.5 Kbp *Sma* I/*Pst* I fragment excised exclusively within LTR regions of ecotropic AKV genomes. The probe, pHR7, is a subclone in pBR322 which contains a 1.6 Kbp *Eco*RI/*Hin*d III insert derived from the leftword end of mouse VL30 clone BVL-1 and includes one copy of the LTR. The autoradiogram is deliberately overexposed to clearly show the 0.5 (LTR) and 0.4 Kbp fragments.

under identical conditions.

A deliberately over-exposed autoradiogram illustrated in figure 5, conclusively demonstrated a lack of hybridization to the 0.5 Kbp *Sma* I/*Pst* I AKV LTR fragment, while the expected non-LTR, 1.4 Kbp *Sma* I fragment was clearly visible. When the same restriction enzyme digests of AKV were blot-hybridized in parallel with the homologous AKV probe, the expected

VL30 CLONE BVL-1 TGAAAGACCCTCGAGGGGAGACCCTTAC
AKV CLONE pAKR59 TGAAAGACCCCTTCATAAGGCTTAGCCA

Figure 6. Nucleotide sequence of the left terminus of the VL30 element of clone BVL-1 compared to the sequence of the initial 28 nucleotides of the 3'LTR of an AKV provirus as determined by Lenz et al. (31).

0.5 Kbp LTR as well as the 0.4 Kbp ecotropic-specific (26) fragments were clearly visible. This reduces the possibility that failure of small fragments to adequately transfer might account for the lack of detectable hybridization to the 0.5 Kbp LTR fragment using pHR7 as a probe.

Homology Between VL30 and MuLV Provirus Inverted Terminal Repeats

In spite of the lack of detectable cross-hybridization, the existance of short regions of sequence homology within the LTRs could not be discounted. Numerous studies have demonstrated that the LTRs of a variety of eukaryotic transposable elements and proviruses are themselves terminated in short inverted repeats of varying length and sequence homology (reviewed in 27 and 28). These sequences most likely facilitate integration of the mobile element into the host chromosome since the last two nucleotides at each terminus are removed, presumably by site specific endonuclease cleavage, during integration (29,30). The resulting 5' and 3' termini of all integrated proviral DNAs and many transposable elements are always TG and CA respectively (27).

Figure 6 presents a short nucleotide sequence corresponding to the left hand terminus of the VL30 element depicted in the physical map of BVL-1 and compares this sequence to the initial 28 nucleotides of the 3' LTR of an AKV provirus as determined by Lenz et al. (31). The first 11 nucleotides of the AKV sequence, TGAAAGACCCC, correspond exactly to an inverted terminal repeat sequence present in the LTRs of all murine leukemia and murine sarcoma proviruses thus far examined (32-35). The VL30 LTR was found to be homologous in the first 10 positions, differing only in the last nucleotide. Thereafter, the two LTRs appear to diverge completely, exhibiting only one homologous position in the next 17. Although several partial homologies have been found in the remainder of the two LTR sequences (unpublished data) they are obviously not sufficient to promote cross-hybridization and their significance is open to question.

VL30-Specific Sequence Representation in Cloned Representatives of Endogenous, Presumably Xenotropic, MuLV Proviruses

When Southern blot analyses were performed using a subgenomic 0.4 Kbp *Sma* I ecotropic-specific fragment from the AKV *env* gene region (26) as a

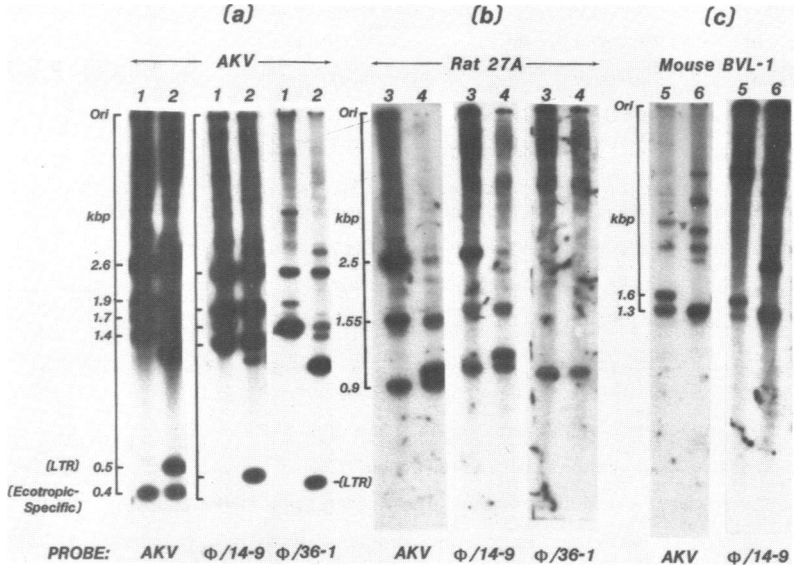

Figure 7. Homologous VL30-related sequences in ecotropic and endogenous xenotropic MuLV proviruses. Southern blots of a) AKV, b) 27A, and c) BVL-1 restriction enzyme digests were hybridized with ^{32}P-labeled probes AKV, λ phage 14-9, or λ phage 36.1 DNA as described in the Methods. Lanes 1) *Sma* I, 2) *Sma* I/*Pst* I, 3) *Eco*RI, 4) *Eco*RI/*Hin*d III, 5) *Hin*d III, and 6) *Hin*d III/*Xho* I. These blots originated from separate gel electrophoretic runs and hence approximate size of various restriction enzyme fragments in Kbp is indicated in the figure. The autoradiograms were deliberately overexposed to clearly show the 0.4 and 0.5 Kbp fragments.

probe, no hybridization was observed to any fragment derived from clone BVL-1 or clone 27A (data not shown). These results indicated that the observed sequence homology between AKV and VL30 genes (Figs. 1 and 3) may not be restricted to ecotropic MuLV's, but might include xenotropic MuLV's as well. To test this hypothesis, we carried out similar Southern blot analyses with endogenous MuLV clones, 14-9 and 36.1, which were originally isolated by Roblin et al. (17) from a C3H/HeN mouse genomic library. The bulk of the evidence, including the presence of several xenotropic-specific restriction sites, strongly suggest that these two clones represent 5' and 3' portions, respectively, of endogenous xenotropic MuLV proviruses (17). These clones do share some overlapping sequences at the junction of *Eco*RI site (17,36). Southern blots of AKV, 27A and BVL-1 restriction enzyme digests were hybridized with ^{32}P-labeled AKV, 14-9, or 36.1 probes.

Figure 7a shows that most of the *Sma* I and *Sma* I/*Pst* I fragments, which span almost the entire AKV genome including LTRs, hybridized to the endog-

enous MuLV clones 14-9 and 36.1. No detectable homology was observed, however, between the 0.4 Kbp *Sma* I ecotropic-specific fragment of AKV and the endogenous MuLV clones. These findings strongly support the conclusion that these endogenous MuLV clones belong to the class of AKV-related xenotropic proviruses in the mouse genome.

It is important to note that essentially the same set of restriction fragments derived from rat and mouse VL30 clones, 27A (Fig. 7b) and BVL-1 (Fig. 7c), respectively, hybridized to both the ecotropic AKV and the xenotropic MuLV clones, 14-9 and 36.1. For example: when an *Eco*RI digest of rat VL30 clone 27A was probed with either AKV or 14-9 DNAs, the same set of restriction fragments, 2.5, 1.55, and 0.9 Kbp, showed up in the autoradiogram represented in figure 7b. It is consistent to note that the 0.9 Kbp fragment being derived from 3'-end of clone 27A was likewise hybridized to the 3'-end MuLV clone, 36.1. Similarly, the 1.6 and 1.3 Kbp *Hind* III fragments of mouse VL30 clone BVL-1 were hybridized to both AKV and the 5'-end derived xenotropic MuLV clone, 14-9. No sequence homology was detected when a *Hind* III digest of BVL-1 was probed with the 3'-end MuLV clone, 36.1 (data not shown).

Based on these findings, we conclude that cloned representatives of both mouse and rat VL30 elements share homology to endogenous MuLV proviruses of both ecotropic and xenotropic host range specificity. Within the confidence limits imposed by the lengths of cross-hybridizing restriction fragments, these homologous regions most likely correspond to the same sets of sequences.

DISCUSSION

The results of these studies contrast with previous reports of a lack of sequence homology between VL30 genes and any of the known ecotropic or xenotropic retroviruses (1,7,8). A probable explanation for this apparent discrepancy is the fact that we employed moderately stringent hybridization conditions in our Southern blot analyses. Indeed, mouse clone BVL-1 was originally identified as VL30 partially on the basis of its failure to hybridize to a cloned AKV provirus using more stringent hybridization conditions (2).

The mouse genome is known to contain 15-30 copies of sequences related to replication-competent MuLV retroviruses of both ecotropic and xenotropic host range specificity (37). Molecular cloning and restriction endonuclease mapping suggests that the bulk of these sequences represent endogenous xeno-

tropic MuLV proviruses and are organized into a relatively small number of classes (17,36,38). The discovery of genetic relatedness between MuLV proviruses and VL30 elements considerably expands the known number and diversity of MuLV-related genes in the mouse genome and raises new questions regarding the origins and evolution of this multigene family.

These data may be interpreted in terms of any of several models ranging from a MuLV-protovirus role for VL30 sequences to the view that VL30 sequences represent extensively deleted and substituted xenotropic MuLV genomes. An alternative, though not mutually exclusive, hypothesis is that VL30 genes and MuLV proviruses have separate evolutionary origins but have undergone genetic recombination events during their evolution. The patterns of sequence homology observed between rodent VL30 genes and MuLV proviruses are not consistent with uniform divergence by single base changes. Rather, the general interspersion of conserved and nonconserved sequences is strongly suggestive of a mechanism involving sequence rearrangements and recombination events. Ample opportunity for the occurrence of such events would be afforded by the copackaging into mixed pseudotype virions, and subsequent reverse transcription, of both VL30 and MuLV or other progenitor RNAs. Although recombination events at the DNA level cannot be ruled out, successive cycles of reverse transcriptase-mediated recombination and reintegration would select for variants able to be more efficiently packaged as well as provide a mechanism whereby the recombinant sequences would be dispersed throughout the mouse genome. A strong selection pressure to utilize a common integration mechanism may well account for the near perfect conservation of MuLV inverted terminal repeats in a VL30 LTR.

Although it cannot be determined with certainty that MuLV clones 14-9 and 36.1 represent xenotropic host range proviruses, the bulk of the evidence suggests that they do (17, Fig. 7). Based on studies of their distribution in feral subspecies of *Mus musculus*, both xenotropic MuLV proviruses and VL30 sequences appear to be of greater evolutionary age than ecotropic viruses related to the AKV-type MuLV (6,38,39). The presence of a similar set of VL30-related sequences in both an ecotropic and xenotropic provirus therefore suggests that the putative recombination events between VL30 and other MuLV-progenitor sequences occurred prior to the emergence of the ecotropic AKV-type viruses some 1 to 2 million years ago. This interpretation is consistent with the depressed Tm of VL30-AKV hybrids although we cannot be certain that this is entirely reflective of sequence divergence and does not include a contribution from short regions of base pairing.

The persistent suggestion that certain sequences present in the AKV provirus genome are more closely related to rat clone 27A rather than to the mouse VL30 clone used in these studies, merits comment. Since we have examined only one cloned representative from each species, these results may simply reflect interclonal variation among related members of multigene families rather than a species-specific relationship. Alternatively, these results may indicate the occurrence of interspecies recombination events during MuLV evolution. Such interspecies recombinations have occurred during passage of Moloney MuLV and Kirsten MuLV through rats, leading to the independent derivations of the Harvey and Kirsten strains of murine sarcoma viruses. These viruses are composed of parental MuLV sequences, a short region of rat DNA encoding the p21 transforming protein, and a large portion of a rat VL30 gene closely homologous to rat clone 27A (10-12, 16). The presence of a rat VL30 gene in two independently derived recombinant sarcoma viruses suggests an unusual propensity for MuLV-VL30 recombination. If the parental Moloney and Kirsten MuLVs are similar to the AKV and MuLV provirus clones 14-9 and 36.1 in containing blocks of VL30-related sequences, these recombinations may have been facilitated by partial sequence homologies between the parental MuLV and rat VL30 genes. Previous heteroduplex mapping studies have, in fact, suggested that a short region of the Harvey murine sarcoma virus which is derived from the parental MuLV has homology to rat VL30 sequences (15).

The results of the present study are strikingly similar to those of a recent analysis of the relationship between intracisternal A particle (IAP) genes and an endogenous retrovirus (M432) of *Mus cervicolor* (40). Like VL30 genes, the IAP genome is present in all species of the genus *Mus* examined thus far (41). The M432 group of retroviruses, however, are apparently restricted to *M. cervicolor* and a closely related species, *M. cooki*, indicating that they are relatively recent additions to the genus. Callahan et al. have recently shown that the M432 genome contains a limited subset of IAP sequences organized into two discrete regions of homology (40). As was found in the present study, these homologous regions did not include significant portions of the LTRs. The data led these authors to suggest that the replication competent M432 retrovirus is a recombinant composed of IAP sequences and sequences derived from another unidentified genetic element. The similarities with the present data, coupled with the greater evolutionary age of xenotropic MuLV, argues that this situation does not represent an isolated case but may be a relatively

recent example of a mode of retrovirus evolution which is quite ancient at least as far as rodents are concerned. Novel genetic recombinations between transposon-like sequence elements may prove to be a common mechanism underlying the evolution of retroviruses.

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