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Structure, Distribution, and Expression of an Ancient Murine Endogenous Retroviruslike DNA Family

MASANORI M. OBATA† AND ARIFA S. KHAN*

Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

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An endogenous retroviruslike DNA, B-26, was cloned from a BALB/c mouse embryo gene library by using a generalized murine leukemia virus DNA probe. Southern blot hybridization and nucleotide sequence analyses indicated that B-26 DNA might be a novel member of the GLN DNA family (A. Itin and E. Keshet, J. Virol. 59:301–307, 1986) which contains murine leukemia virus-related *pol* and *env* sequences. Northern analysis indicated that B-26-related RNAs of 8.4 and 3.0 kilobases were transcribed in thymus, spleen, brain, and liver tissues of 6-week-old BALB/c mice.

Several distinct classes of retroviruslike elements exist in the mouse genome (8). We have previously cloned and characterized different endogenous murine leukemia virus (MuLV)-related sequences that are present in the BALB/c mouse genome (16). The cloned DNAs were divided into two subclasses based upon their degree of homology to known MuLV proviruses: about half were closely related to MuLVs in their long terminal repeat (LTR), *gag*, *pol*, and *env* regions, whereas the remaining DNAs were quite divergent from MuLV DNA. Extensive studies have previously been carried out on endogenous retroviral sequences which are closely related to known ecotropic, xenotropic, and mink cell focus-forming (MCF) MuLVs. In this report we describe the structure, transcripts, and genomic distribution of an endogenous MuLV-related DNA, B-26, which is distantly related to known MuLVs.

B-26 DNA was previously cloned from a BALB/c mouse embryo gene library by using a generalized MuLV DNA probe, ^{32}P-labeled MuLV$_{gen}$ (16), under normal-stringency hybridization conditions (melting temperature [T_m] = -10°C, using the formula $T_m = 16.6 \log(\mathrm{Na}^+) + 0.41 (\%\mathrm{G+C}) + 81.5$ [22]). The maximum extent of MuLV-reactive sequences present in λB-26 DNA was determined by low-stringency hybridization (T_m, -49°C) of digested DNA fragments with ^{32}P-labeled MCF13 MuLV DNA, a full-length MuLV provirus (27). The 15-kilobase (kb) cloned DNA segment contained at most 6 kb of MuLV-reactive sequences located at the 5′ end of the insert. The location of the reactive sequences in the B-26 provirus is shown in Fig. 1. The colinearity of B-26 retroviral sequences with MuLV DNA was determined by normal-stringency hybridization (T_m, -10°C) by using various subgenomic MuLV DNA probes spanning the *gag*, *pol*, and *env* regions (data not shown). B-26 DNA did not react with ^{32}P-labeled 0.9$_{Bgl\text{-}Bam}$, a *gag*-specific probe (16), and B-26$_{\mathrm{LTR}}$ probe at its 5′ end because of truncation of the provirus resulting from the presence of the cloning site. B-26 DNA hybridized with 1.9$_{Bam}$, 2.7$_{Sal\text{-}Bam}$, and 1.2$_{Kpn}$ DNA probes (16) which encompassed MuLV *gag-pol*, *pol-env*, and *env* regions, respectively. Interestingly, both xenotropic and ecotropic MuLV *env*-specific DNA probes, ^{32}P-labeled X$_{env}$ (3) and ^{32}P-labeled Ec$_{env}$ (4), respectively, hybridized with B-26 DNA, suggesting an MCF-like *env* gene (16). The restriction map of B-26 proviral DNA was determined and is presented in Fig. 1. It should be noted that, although MuLV DNA probes hybridized with B-26 DNA, no heteroduplexes were formed between the latter DNA and AKR ecotropic MuLV provirus under conditions requiring 75% sequence homology (data not shown).

Certain regions of the B-26 provirus did not hybridize with any MuLV DNA probe (Fig. 1). To characterize these non-MuLV-reactive regions that were present in B-26 DNA, other endogenous murine retroviruslike DNA probes were used in Southern blot hybridization studies carried out under normal-stringency conditions. These included pM1A4, which contained the A-particle gene (kindly provided by K. Lueders [17]), and VL30 DNAs p3/I and p3/IV (kindly provided by E. Keshet [14]). B-26 DNA hybridized with only the VL30 DNA probes. The p3/I DNA probe reacted weakly with the 1.4-kb *Eco*RI segment located at the 5′ end of *pol*, and the p3/IV DNA probe hybridized strongly with the 3.6-kb *Eco*RI segment, which contained the rest of the B-26 *pol* and *env* sequences (data not shown). The VL30-reactive sequences in B-26 DNA were not localized further.

Nucleotide sequencing of B-26 DNA in the region residing 3′ of the MuLV-reactive sequences indicated a retrovirus-like LTR structure (Fig. 2) (28). All the transcriptional regulatory signals were conserved. An additional CCAAT box was present at nucleotides 199 to 203. B-26 LTR sequences were compared under low-stringency conditions with those present in the Genebank Release 40.0 (February 1987) by using the SEQFN program obtained from the Molecular Biology User's Group, National Institutes of Health. The results indicated a high degree of nucleotide sequence homology (91%) with the GLN-3 LTR; 71 and 70% base homologies were seen with NZB xenotropic and Moloney (Mo) ecotropic MuLV LTRs, respectively; and 78% base homology was seen with the VL8 LTR. Recently, several protein-binding sites (LV$_a$, LV$_b$, and LV$_c$) have been localized in the enhancer element of MoMuLV (26). The LV$_a$ site was conserved in B-26 (nucleotides 101 to 106); another site similar to LV$_a$ except for a 1-base-pair change was also present in the B-26 sequence upstream of the LV$_a$ site (nucleotides 91 to 96). A mutated LV$_b$ site was present in the B-26 LTR (nucleotides 109 to 115), with a single base

* Corresponding author.

† Present address: Laboratory of Genetechnology and Safety, the Institute of Physical and Chemical Research, Tsukuba Life Science Center, 3-1-1 Koyadai, Yatabe, Tsukuba, Ibaraki 305, Japan.

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LTR        gag              pol                 env          LTR

                X Bg P  R P           Bg  Sm  S        R  X  S
a
b
c               A                     B                   C

0      1      2      3      4      5      6      7      8      9 kb
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FIG. 1. Structure of B-26 provirus. Restriction map and maximum region of MuLV-reactive sequences are shown on line a. Restriction sites were mapped by Southern blot hybridization under normal-stringency conditions by using ^{32}P-labeled MuLV subgenomic DNA probes (see text). The filters containing B-26 DNA fragments were hybridized overnight at 60°C in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10× Denhardt solution–0.1% sodium dodecyl sulfate–50 μg of denatured salmon sperm DNA per ml and washed at 60°C in 0.1% sodium dodecyl sulfate–0.1× SSC for 30 min. Restriction site abbreviations: R, *Eco*RI; X, *Xba*I; Bg, *Bgl*II; P, *Pst*I; Sm, *Sma*I; S, *Sac*I. Restriction sites going through the bar, representing the B-26 provirus, were used to locate the maximum extent of MuLV-reactive sequences. To achieve this, filters containing B-26 DNA fragments were hybridized with ^{32}P-labeled MCF13 MuLV DNA under low-stringency conditions at 50°C in 6× SSC and washed at 50°C in 6× SSC. The hybridization signal is indicated as strong (▨), weak, (◺), or none detected (□). Line b shows the region at the 3′ end of the provirus that was sequenced. The location of B-26-derived DNA probes (described in the text) is indicated in line c. Abbreviations: A, B-26$_{pol}$; B, B-26$_{pol\text{-}env}$; C, B-26$_{LTR}$.

```
                                                     50
B-26    TAAAAGA   A AATAAAACTT GAGACCTGTGATTCATGTAATG TATGT  CAAATAGC          CCAAA  GAGTTGT TGTGAGC TTTGAAA
GLN-3   .   .     . ..GG...   . A...  ....A............ .....  T.......          .....  .......T....... .......
VL8                                                  ..   .T.. ..            ..CC            .. ..   ...G..
Xeno    .G.....CCCC.CC....GG...A.CA.G..A .C.G..  ....C.CC..T.TG...  G..ATGAAAAAGTA...     ...C       ....T ...C...
MoMLV   .G.....CCCC.CCTGT.GGT..G.CA.G..A .C..A.  ....C.CC..T.TG...  G..ATGGAAAAATA.AT..CT...AA .   A...AAG..C.G.

                            (LVa)    100   LVa      (LVb)            LVc
B-26    C               CTGGGG CT GAG  AACATAG CA  GAACAG ACCAGGACATG CCCAGG CA     GGCCCAT TG     CTTCCCTAGC  TCC
GLN-3   .               T...... .. ...  ....C.. ..  ...... ..T...... .T...G.. ..     A...... CA     .CA... ...  ...
VL8     .ATT C          .....AA.    ..TC..  ..CTTA.  ...T.A . G.... .. TG ...A..TAACA..G.T..C..AACCGA...AAC.A.  ..A
Xeno    AGTTACAAAAAAGT.T..  T.AA..  ..T. ..GTT   ...T.AT..TG.....G.GG...AA ..      .. AT..C..       .GGT.G...  A..
MoMLV     T             .AA..T . A. .  ....  .  .TG......   .T.A.T...GG...AA ..      .. AT..C..       .GGT A.'..AG.T.
                                                 LVa                          LVb                               L
      150
B-26    CA  CCCCT CTGA   CCTA AG
GLN-3   ..  ..... ....   .TC. ..
VL8     ..G  AA..  ....CAC...GC.T
Xeno    TGGG....GG..C.GGG.. . ..AACAGATGG
MoMLV   .TG ....GG..C.GGG.. . ..AACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACA
         Vc                    LVa                          LVb                    LVc
                                                                                    (LVa)
B-26                                                              TT   AAA TGTTAACAGG CTGCTGATGTTTAAATGGAC
GLN-3                                                             ..   ... ..... .... .................A..
VL8                                                               G.   .C. ... ..TTTTT............G...AAG.
Xeno         TACTCAGATAAAGCGGAACCAGC    AACAGTTTCTGAAAAAGTCCCACCTCAG  ..TC ..   ...CC.C    AAA...CCAGG....ACC.
MoMLV   GATGGTCCCCAGAT   GCGGT CCAGCCCTCAGCAGTTTCT AGAGA  ACCA  TCAGATG..TCC.GGG.. CCC..A.      ..CC .G..... ..

     200                                                                                          250
B-26    CAA       TTATGTGAAACC   GCACCAATT                             TCC  TCCCCC AGTCCCTCTCCTTTTCT ATAAAAAC C
GLN-3   ...       .C..........   ..G.....                              ..  ...... ..C...A.........  ......... .
VL8     ...       .AG.....C..ATT..TG ....                              ..ACA....TA.. ..    ....AC..C....... ..
Xeno    ...   GCC....T.....T   A.....CAGCTCGCTTCTCGCTTCTGTACCCGCGCTTTT.G.  .....  ..C...AG ..      . ......GGG
MoMLV   .CTGTGCC....T.....T   A.....CAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTC .G.  .....G .. .          ..A ......GAG.

                                                    300
B-26    TCT GACTTCCAAGCCTCGTGGTCGA TTCTACTGTC TCC  T GT   GTGAGAT  ACGTTTC   AACCC   G G AGCTCCGCCATTAAAATACCT
GLN-3   C.. AG.....................A..C...... ...  . ..TGT.......  .......   G....   . . ........ ......A....
VL8     C..A.G...T G.......A..CT..CAA.C.T.A.T ...  . ..   ...G...GC.T...  GGTC...AGCTCT.  .....T.T......C.....
Xeno    .AA ..AC... .CA.. . .. ..    .GC.A...A...GA.A.A   C.... .   ..CC .GGGT....   .T.    .T. ..A.... G...
MoMLV   C.A C.ACC.. TCA.... ..G..    .C.  ...C...GA.T.A   C.... .   ..CC .GGGT....   .T.T.  .   ..A....CCCT..
                      ←U3|R→
          350                                               400
B-26    TGTGTTGTT ACATCAAGGTGTTGTGTTCTATTCG TGATT CTTGGGTG CACGCCGAATTGGGAGTTGAGTGGGGGTTTCCC  CA CTAGGTTCTTTCA
GLN-3   CT....... ..........C............... C.......... .A ...T....C....C.............. ..  ..GA.........
VL8     C....AA.. ............C.. . .. . .....TT....... .C.A.....C...A.........A...... C.. ..........A...
Xeno    .T..C....TG....CGAA  .C...G..  ...C....C ......A.GGT.T..TC.GA.T.. ....C.       A...AG.T .GG..G.......
MoMLV   ..  CA... G.....C  .AC.....G...  ..C.. ..C......A.GGT.T..TCTGA.T.. ....C.       A...GT..G.GG..G.......
              ←R|U5→
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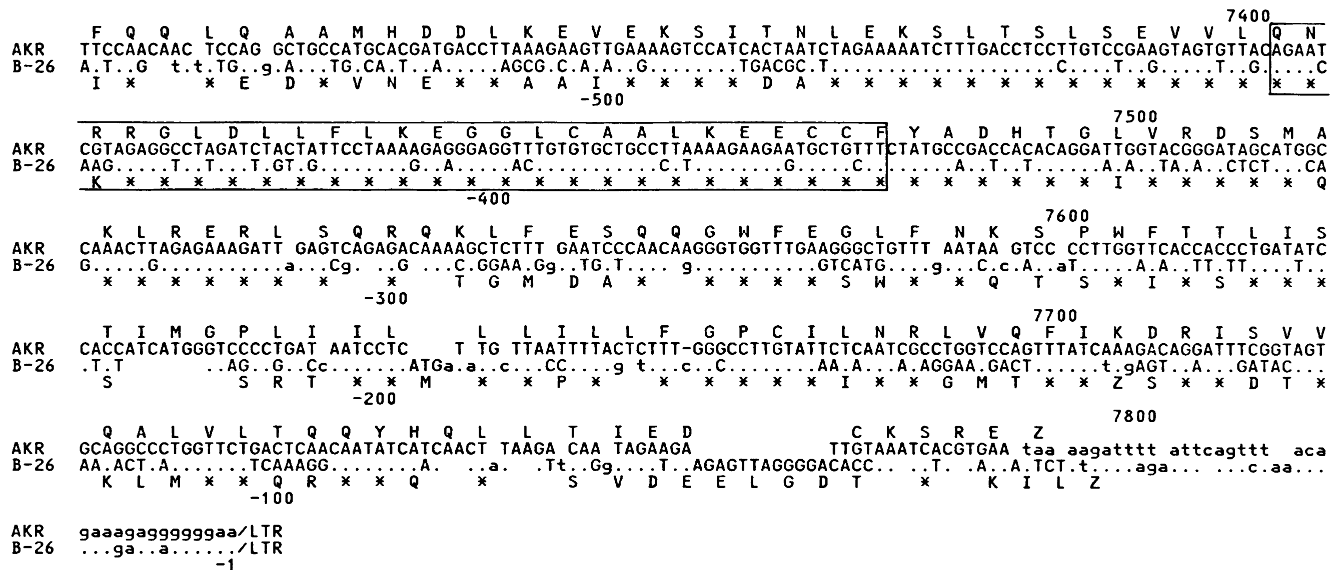

FIG. 3. Nucleotide and deduced amino acid sequences of 3′ *env* region of B-26 DNA. B-26 and AKR ecotropic (AKR) (11) MuLV DNAs in the p15E region are compared. Map positions of nucleotides from the 5′ terminus of AKR RNA are shown above the sequence; in the case of B-26, the nucleotide positions from the 3′ LTR, shown below the sequence, are indicated. Dots indicate identical nucleotides, and asterisks indicate identical amino acids. Different bases and amino acids are indicated. The boxed region encompasses p15E sequences which have been reported to be involved in immunosuppression (7). Gaps were introduced to attain the greatest sequence homology. Lowercase letters shown in the nucleotide sequence of B-26 were not included in the amino acid translation. The longest open reading frame is present between nucleotides −529 and −311; the maximum amino acid homology outside this sequence stretch was achieved by artificial alignment by using corresponding nucleotides as codons.

change at nucleotide 114. The inverse sequence of LV_c was present in the B-26 LTR at nucleotides 123 to 127. The spatial arrangement of these sites, however, was similar in B-26 and MoMuLV LTR sequences.

Nucleotide sequences at the 3′ end of *env* of B-26 could not be aligned by the computer, even under low-stringency conditions, with any known retrovirus sequences. Translated amino acids, however, could be visually aligned in analogous regions at the 3′ end of *env* of B-26 and AKR ecotropic MuLV DNAs (Fig. 3). A 26-amino-acid sequence which was located in the p15E region and that has been implicated in immunosuppression (6, 7) was conserved in B-26 DNA (nucleotides −441 to −366) with 1 amino acid change (nucleotides −436 to −434).

To confirm that the sequences present in B-26 provirus were not rearranged because of molecular cloning, BALB/c DNA was analyzed by using B-26-derived DNA probes under high-stringency conditions (T_m, −2°C), which eliminated cross-reactivity with endogenous MuLV- and VL30-related sequences. B-26 DNA probes included B-26_{pol}, which consisted of a 1.4-kb *Eco*RI segment containing 5′ MuLV-related *pol* sequences; B-$26_{pol\text{-}env}$, which consisted of a 3.7-kb *Eco*RI segment containing the 3′ half of MuLV-related *pol* sequences and the 5′ two-thirds of the *env* region; and B-26_{LTR}, which consisted of a 255-base-pair *Hin*fI fragment derived from the 3′ LTR region. The map locations of these DNA fragments are shown in Fig. 1. Southern blot hybridization of *Sac*I-digested DNA indicated that all of the fragments which reacted with ^{32}P-labeled B-26_{pol} DNA also hybridized with ^{32}P-labeled B-$26_{pol\text{-}env}$ and ^{32}P-labeled B-26_{LTR} DNAs (data not shown).

The distribution of B-26-specific sequences in rodent DNAs was studied by using a ^{32}P-labeled B-$26_{pol\text{-}env}$ DNA probe under high-stringency hybridization conditions. Southern blot analysis of *Hin*dIII-digested DNAs indicated a high degree of conservation of B-26-related sequences in all inbred mouse DNAs (data not shown). Southern blot analysis of *Pst*I-digested wild mouse and other rodent DNAs is shown in Fig. 4A and B, respectively. DNAs of the four rodent families *Muridae*, *Cricetidae*, *Sciuridae*, and *Caviidae* were analyzed. All wild mouse DNAs (family *Muridae*, genus *Mus*) contained several strongly reactive fragments (Fig. 4A). The copy number and hybridization signal of B-26-related DNA fragments appeared to be similar in all DNAs of the mouse subgenus *Mus* (Fig. 4A, lanes 1 to 9). Several *Pst*I segments were conserved in the five different *Mus musculus* subspecies (Fig. 4A, lanes 1 to 5). At least three fragments, of 12.0, 4.5, and 0.6 kb, were present in all the DNAs. Evolutionarily distant mice such as *Mus shortridgei* (subgenus *Pyromys*; Fig. 4A, lane 10) and *Mus pahari* (subgenus *Coelomys*; Fig. 4A, lane 11) contained fewer copies of B-26-related sequences. No detectable *Pst*I fragment was conserved in all of the mouse DNAs.

Multiple copies of B-26-related sequences were also de-

FIG. 2. Nucleotide sequence analysis of B-26 LTR. LTR sequences of B-26 provirus were determined by the method of Maxam and Gilbert (18) and were compared with LTRs of GLN-3 (13), VL8 (12), NZB xenotropic MuLV (xeno) (19), and Mo ecotropic MuLV (MoMuLV) (24) DNAs. Base alignment between these LTRs was achieved visually to attain maximum sequence homology. Gaps were introduced to attain maximum sequence homology. Dots indicate identical bases, arrows indicate inverted repeats, and boxes indicate regulatory signals. Different bases are indicated. Protein-binding sites unique to MoMLV (LV_a, LV_b, and LV_c) (26) are underlined. The MoMuLV protein-binding sites conserved in B-26 are indicated; mutated sites are given in parentheses; an inverted sequence is indicated by a broken line.

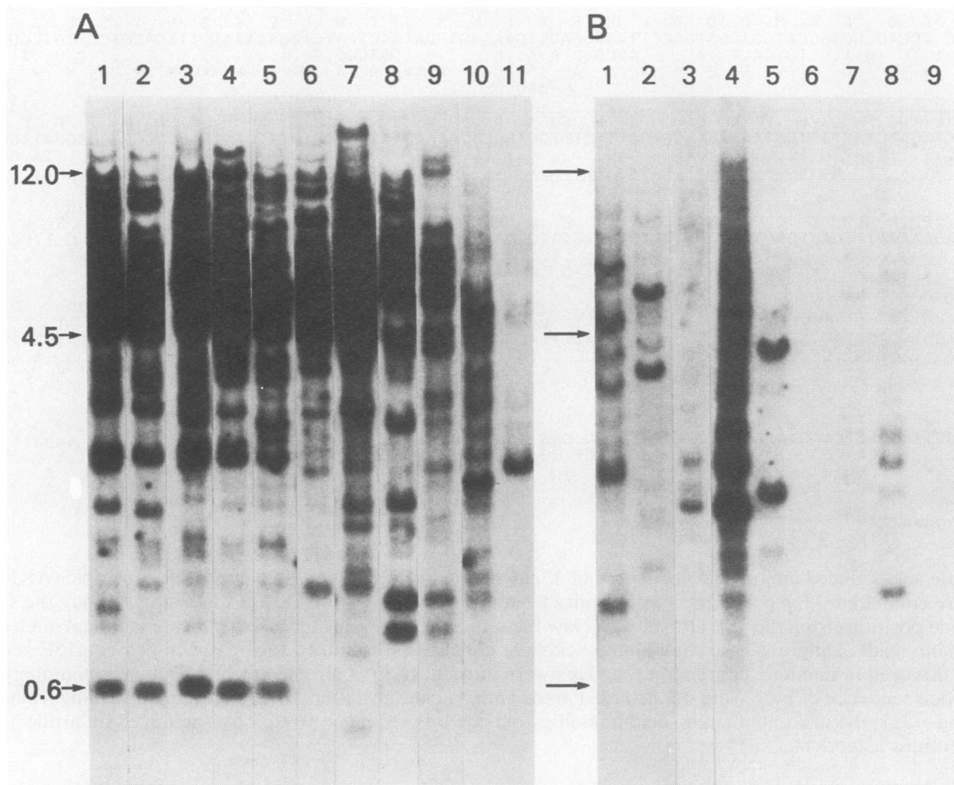

FIG. 4. Distribution of B-26-related sequences in rodent genomes. *Pst*I-cleaved DNAs were electrophoresed on 0.8% agarose gels and transferred to nitrocellulose filters (25). Southern blots were hybridized with ^{32}P-labeled B-$26_{pol\text{-}env}$ DNA under high-stringency conditions at 68°C in 2× SSC–10× Denhardt solution–0.1% sodium dodecyl sulfate–50 μg of denatured salmon sperm DNA per ml, and the filters were washed at 68°C in 0.1% sodium dodecyl sulfate–0.1× SSC 3 times for 15 min each time. Wild mouse DNAs (A) included *M. musculus* subsp. *domesticus*, *M. musculus* subsp. *poschiavinus*, *M. musculus* subsp. *musculus*, *M. musculus* subsp. *castaneus*, *M. musculus* subsp. *molossinus*, *M. spretus*, *M. hortulanus*, *M. cervicolor*, *M. cookii*, *M. shortridgei*, and *M. pahari* (lanes 1 to 11, respectively). Other rodent DNAs (B) included those from rat, Chinese hamster, woodchuck, ground squirrel, gerbil, guinea pig, deer mouse, and Syrian hamster (lanes 1 to 8, respectively). Human DNA is present in panel B, lane 9. The autoradiograms shown in panels A and B were exposed for 15 and 120 h, respectively. Molecular sizes (in kilobases) of some of the conserved fragments are indicated.

tected in all other rodent genomes (Fig. 4B); however, the autoradiogram had to be exposed about 7 times longer to achieve the same hybridization intensities seen in wild mouse DNAs (Fig. 4A). The number of copies of B-26-related genes in the rat genome (family *Muridae*, genus *Rattus*; Fig. 4B, lane 1) was greater than that in DNAs of the other rodent families (Fig. 4B, lanes 2 to 8). Genomes of the Chinese hamster, gerbil, and Syrian hamster (family *Cricetidae*) contained a few strongly hybridizing fragments along with other weakly reactive bands (Fig. 4B, lanes 2, 5, and 8, respectively), whereas DNA of the deer mouse (also of the family *Cricetidae*) contained only weakly hybridizing *Pst*I fragments (Fig. 4B, lane 7). DNAs of rodents of evolutionarily distant families also contained B-26-related sequences. At least two *Pst*I fragments were seen in woodchuck DNA (family *Sciuridae*; Fig. 4B, lane 3), and some weakly hybridizing sequences were present in the guinea pig genome (family *Caviidae*; Fig. 4B, lane 6). The high copy number of ^{32}P-labeled B-$26_{pol\text{-}env}$-reactive sequences in ground squirrel DNA (family *Sciuridae*; Fig. 4B, lane 4) could be due to its 50% larger genome compared with those of other rodents. B-26-related sequences were not detected in human DNA (Fig. 4B, lane 9) even under low-stringency hybridization conditions (data not shown).

The various rodent DNAs that were analyzed for B-26-related sequences were also hybridized with the ^{32}P-labeled MCF13 MuLV DNA probe under high-stringency conditions. Analysis of *Eco*RI-digested DNAs indicated that the genomic distribution of MuLV-specific sequences was distinct from that of B-26-like genes, and there were fewer and less-conserved MuLV-reactive fragments (data not shown).

Northern blot analyses were performed on total cellular RNAs isolated from thymus, spleen, brain, and liver tissues of 6-week-old BALB/c mice by using ^{32}P-labeled B-$26_{pol\text{-}env}$ DNA (Fig. 5A) and on poly(A)$^+$ liver RNA by using ^{32}P-labeled B-$26_{pol\text{-}env}$ and ^{32}P-labeled B-26_{LTR} DNA probes (Fig. 5B and C, respectively). Hybridization was carried out under high-stringency conditions to eliminate cross-reactivity with MuLV transcripts. RNA species of 8.4 and 3.0 kb were detected.

The results presented here show that the B-26 provirus is a chimeric DNA containing GLN-related sequences in the LTR and VL30- and MuLV-related sequences in the *pol* and *env* regions. Hybridization studies indicated that the internal B-26-specific sequences were highly conserved in all rodent DNAs and are, therefore, ancient with respect to MuLV and VL30 genes, which were present only in *Mus* genomes (9, 20; unpublished data). It can thus be speculated that B-26 provirus contains an ancestral gene from which MuLV and VL30 sequences have evolved and diverged. Because of the close relatedness of the LTRs (91% homology), B-26 provirus has been tentatively assigned to the GLN DNA family

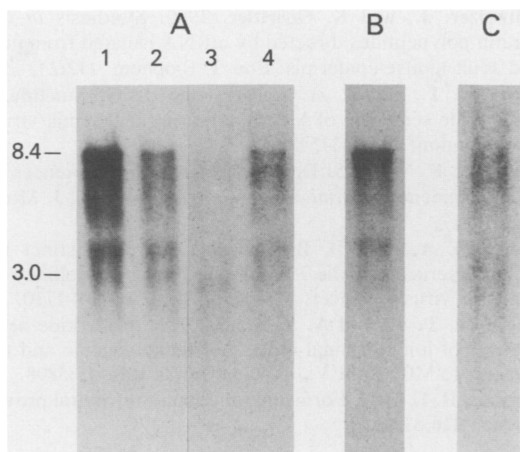

FIG. 5. B-26-related transcripts expressed in BALB/c mouse tissues. Northern blot analyses were performed (15) with 5 μg of total cell RNAs, which were isolated as described previously (5, 23), from thymus, spleen, brain, and liver tissues of two 6-week-old mice (Small Animals Section, National Institutes of Health) by using ^{32}P-labeled B-$26_{pol\text{-}env}$ DNA (A) (lanes 1 to 4, respectively). Poly(A)$^+$ RNA was obtained as described previously (1). Filter strips containing 1 μg of poly(A)$^+$ liver RNAs were hybridized to ^{32}P-labeled B-$26_{pol\text{-}env}$ and ^{32}P-labeled B-26_{LTR} DNAs (B and C, respectively). Hybridization was carried out at 55°C as described previously (15). The filters were washed under high-stringency conditions at 73°C twice for 10 min in 2× SSC with 0.1% sodium dodecyl sulfate, and twice for 15 min in 0.1× SSC with 0.1% sodium dodecyl sulfate, followed by a rinse in 2× SSC. The blot shown in panel A was exposed for 15 h, that in panel B was exposed for 2.5 h, and that in panel C was exposed for 90 h. RNA sizes (in kilobases) were determined by comparison with ethidium bromide-stained RNAs of known sizes present in an RNA ladder (Bethesda Research Laboratories, Gaithersburg, Md.).

(13). It should be noted that, although B-26 and GLN-3 LTRs were closely related, their internal sequences were distinct. Unlike GLN DNA, B-26 contained MuLV-related *pol* and *env* sequences. The exact relationship between these two DNAs remains to be determined.

Genomic distribution studies indicated that B-26-related sequences have existed in the rodent germline for at least 50 million years; these sequences were present in the mouse as well as in evolutionarily distant rodents like the woodchuck and guinea pig (10). In contrast, MuLV-specific sequences were only detected in inbred and *M. musculus* wild mouse DNAs (20; unpublished data). The conservation of B-26-specific sequences in rodents correlated well with their genetic distance from inbred mice, except in the case of the deer mouse (2, 10). The highest copy number, the greatest sequence homology, and the largest number of fragments with similar sizes were seen in the DNAs of inbred and closely related wild mice. Thus, the amplification of B-26-related genes seems to have occurred in the *Mus* genome subsequent to the divergence of mice from rats (about 10 million years ago [21]) and prior to the proliferation of MuLV-specific genes.

B-26-related transcripts (genomic length, 8.4 kb; *env* mRNA size, 3.0 kb) were detected in brain, liver, thymus, and spleen tissues of 6-week-old BALB/c mice. The expression was tissue specific: low in brain and liver and high in thymus and spleen. Interestingly, by using a B-$26_{pol\text{-}env}$ DNA probe, the 8.4-kb RNA species was present in a much greater amount than the 3.0-kb RNAs, whereas both transcripts were present in low and similar amounts when they were examined by the B-26_{LTR} DNA probe. These results indicate that the internal sequences of some endogenous B-26-related proviruses might be associated with LTRs which are diverged from those of B-26 DNA. It remains to be determined whether B-26-related sequences are expressed in other rodents and whether a novel retrovirus is associated with this gene family.

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