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Insertional Activation of N-*myc* by Endogenous Moloney-Like Murine Retrovirus Sequences in Macrophage Cell Lines Derived from Myeloma Cell Line-Macrophage Hybrids

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Hybrids formed from a myeloma cell line, NS1, and macrophages initially show myeloma properties but later, after loss of the parental macrophage genome and consequent loss of myeloma characteristics, express macrophage properties. Molecular studies demonstrated that macrophage properties in the hybridomas originate from the NS1 parental cells (M. Setoguchi, S. Yoshida, Y. Higuchi, S. Akizuki, and S. Yamamoto, Somatic Cell Mol. Genet. 14:427–438, 1988). In such hybrids, N-*myc* was activated by insertion of endogenous Moloney-like retrovirus sequences into mouse N-*myc* exon 3 when the hybrids gained macrophage properties. Interestingly, expression of N-*myc* took place in all aged hybrids. These results suggest that such unique insertional mutagenesis occurs in a regionally specific manner and that expression of N-*myc* may play a role in hematopoietic lineage conversion.

In mouse chromosomes, there are many proviral retroviruses (RTV), which normally behave as part of the host genome. RTV consist of a structural and functional unit and have a transposable nature (5). Sometimes, therefore, RTV are transcribed, reverse transcribed, and reintegrated at other chromosomal sites, functioning as insertional mutagens (22). Infected RTV are known to have a similar potential. Proto-oncogene loci appear to be the preferred integration sites of RTV (6, 34), and overproduction of oncogene products or of aberrant products is thought to cause neoplastic transformation. In addition, activation by transposition of RTV should provide cellular conditions similar to those found in developmental stages of cells, since various oncogenes are expressed in limited stages of development and may be functionally involved in differentiation or development.

It has been reported that N-*myc* has unique features of expression mainly associated with neurogenic tumors such as neuroblastoma and retinoblastoma (26, 40, 41, 49, 58) and with fetal and newborn tissues of mice and pre-B cells but not with mature B and myeloma cells (59). Although N-*myc* has been reported to have nucleotide and amino acid sequences closely related to, and a functional homology with, c-*myc* (11, 23, 27, 50), these features of expression suggest that N-*myc* plays unique roles in neoplastic proliferation and cell differentiation. Gene amplification is a well-documented mechanism of N-*myc* activation (40, 42). However, expression of N-*myc* by RTV insertion has recently been explored in Moloney murine leukemia virus (MoMuLV)-induced T-cell lymphomas (54, 55).

We have studied the properties of cell hybrids formed from a mouse myeloma cell line, NS1, and macrophages from mouse or human species and found that although such hybrids initially lack macrophage functions, they later express them. On the other hand, the κ light-chain-producing ability of the hybrids, typically detectable in NS1 cells and in the hybrids during the early period after cell fusion (8 weeks), is lost once macrophage functions become detectable (44). Furthermore, expression of macrophage function is accompanied by a marked reduction in the number of chromosomes, aneuploidy being observed in most of the macrophagelike cells. Molecular analysis of the expression of parental cell properties of the hybrids indicates that macrophage properties originate from NS1 genes and that various genes markedly expressed in macrophages are transcribed (31, 44). Furthermore, a significant enhancement in c-*fos* expression has been observed in macrophagelike cells (20). In this report, we have documented that these hybrid cell lines show enhanced expression and spontaneous transposition of MoMuLV-like proviral (MLRV) sequences into the N-*myc* locus and expression of N-*myc*. It has been reported that v-*ras*-transformed pre-B-cell lines spontaneously generate cells of macrophage phenotype and morphology (10, 21). Furthermore, a recent report that switching to a monocytic lineage frequently occurs when B-lymphoid tumor cell lines from Eμ-*myc* transgenic mice are infected with v-*raf* (25) strongly suggests that oncogenes play an important role in lineage conversion. We therefore discuss the significance of oncogene involvement in the proliferation and generation of macrophage cell lines.

MATERIALS AND METHODS

Cell lines. HINS and CANS lines were produced by fusion between the 8-azaguanine-resistant myeloma cell line P3/NS1/1-Ag-1 (NS1) and human peripheral blood monocytes and mouse pleural macrophages, respectively, as previously described (44, 57). Murine neuroblastoma cell lines C1300, N18, and N20 were kindly supplied by K. Mifune, K. Koono, and T. Amano. The Rauscher murine leukemia virus-producing cell line JLS-V9 was a gift of N. Yamamoto and N. Kobayashi.

Isolation of MLRV-related cDNA clones. Construction of aged HINS-specific cDNA clones has been described elsewhere (44). Briefly, ^{32}P-labeled single-stranded cDNA was synthesized from aged HINS cell poly(A) mRNA by using avian myelomatosis virus reverse transcriptase (20). The cDNA was hybridized with an excess of poly(A) mRNA from early HINS cells. Single-stranded cDNA fractions

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obtained through a hydroxyapatite column were concentrated and hybridized with an excess of poly(A) mRNA from aged HINS cells. The hybridized cDNA was used for second-strand cDNA synthesis as described by Gubler and Hoffman (18). The second-strand cDNA thus synthesized was inserted into the *Pst*I site of plasmid pBR322 by the G-C tail method. Transformation was performed by the method of Hanahan (19), using *Escherichia coli* N38. ^{32}P-labeled cDNA was synthesized as previously described (44) from poly(A) mRNA of aged and early HINS cells. The library was screened with these probes, and the cDNA clones that hybridized only with the ^{32}P-labeled aged HINS probe were selected.

Plasmids prepared from the nearly 120 clones were ^{32}P labeled by nick translation and subjected to Northern (RNA) blot hybridization analysis, using poly(A) mRNA from aged and early HINS cells. Probes binding weakly or not at all to the latter but hybridizing to the former were selected and classified into a number of groups according to their hybridization profiles.

Nucleotide sequence analysis. The nucleotide sequences of the insert of clone HIC4, from the largest cDNA group, and of genomic clones were determined by the dideoxy-chain termination method after subcloning of various restriction fragments into pUC118.

Northern blot hybridization. Northern blot hybridization was performed as previously described (20). Briefly, total RNA prepared from various cell lines was electrophoresed through a 1.5% agarose–6% (vol/vol) formaldehyde gel and blotted onto nylon membranes. These membranes were exposed to UV for 7 min and then prehybridized and hybridized with a ^{32}P-labeled nick-translated cDNA probe.

DNA polymerase assays. Assays were performed essentially by the method of Poiesz et al. (37). Briefly, virus particles were obtained from cell-free supernatant by using 30% (wt/vol) polyethylene glycol 6000 and disrupted with a Tris–Triton X-100 suspension buffer. A portion of the disrupted virus solution was subjected to a reverse transcription assay, using poly(A) (Pharmacia, Uppsala, Sweden), d(pT) (Collaborative Research, Inc., Waltham, Mass.), 10 mM $MgCl_2$, and [^{3}H]TTP.

Production of genomic libraries and isolation of MLRV-associated clones. The bacteriophage EMBL3-aged HINS cell library was produced by the method of Frischauf et al. (15). Briefly, genomic DNA prepared from aged HINS cells was partially digested with *Mbo*I, followed by size fractionation via sucrose gradient ultracentrifugation. The DNA fragments in the size range of 12 to 20 kilobases (kb) were inserted into the *Bam*HI site of EMBL3 arms. The annealed DNA was packaged by using a commercial packaging extract (Strategene Cloning Systems, La Jolla, Calif.), and the phage were grown in *E. coli* Q359. Phage plaques (10^6) were screened with a ^{32}P-labeled HIC4 probe at 68°C.

Southern blot hybridization. DNA fragments from the N-*myc* locus were prepared from subclones of the MLRV-related genomic clones C4B and HN7 by digestion with restriction enzymes or deletion using exonuclease III and mung bean nuclease (D probe). DNA fragments produced by restriction endonuclease *Bam*HI or *Xba*I were separated by electrophoresis on 1% agarose gels in Tris acetate buffer and then transferred to nylon membranes as previously described (44). The membranes were UV irradiated, prehybridized, and hybridized with ^{32}P-labeled DNA fragments from the N-*myc* locus for 20 h at 42°C in 50% formamide–5× Denhardt solution–5× SSPE (20× SSPE is 3 M NaCl, 0.2 M NaH_2PO_4, plus 20 mM EDTA, pH 7.4)–0.1% sodium dodecyl sulfate (SDS)–100 μg of salmon sperm DNA per ml. They were then washed five times in 0.1% SDS–2× SSC (SSC is 0.015 M NaCl plus 0.015 M sodium citrate) at room temperature and finally washed twice in 0.1% SDS–1× SSC at 68°C.

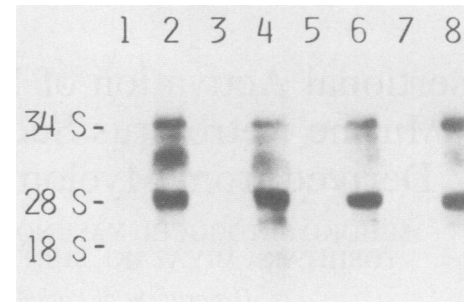
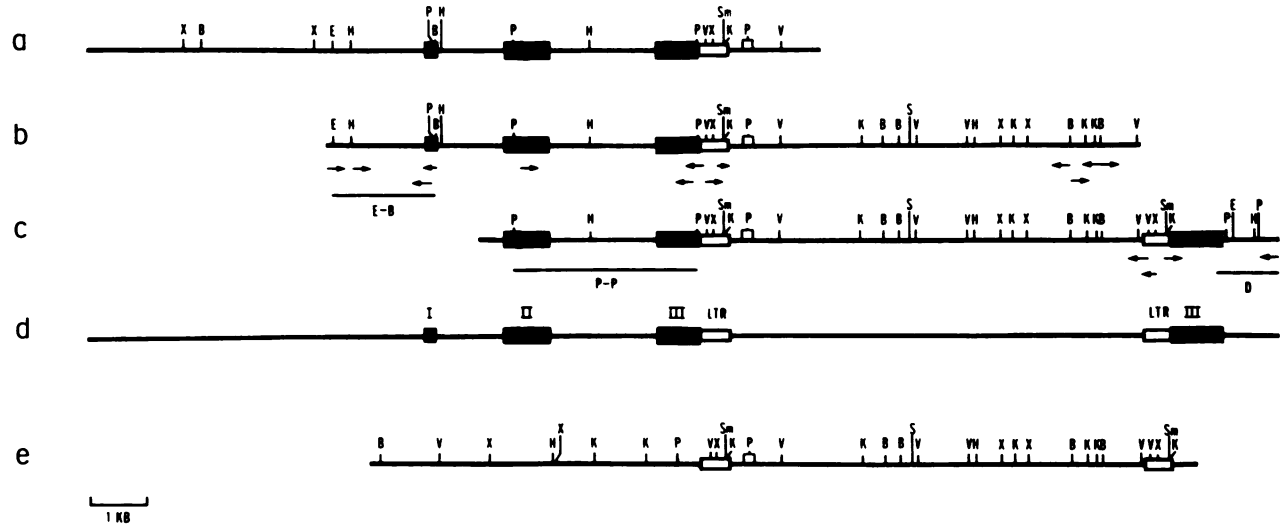


FIG. 1. Transcription of MLRV in aged HINS and CANS cell lines. Total RNA (30 μg) was electrophoresed in a 1.5% formaldehyde gel, blotted on a nylon membrane, and hybridized with a ^{32}P-labeled HIC4 cDNA probe. Lanes contained the following sources of RNA: 1, early HINS-A4; 2, aged HINS-A4; 3, early HINS-B3; 4, aged HINS-B3; 5, early CANS-20; 6, aged CANS-20; 7, early CANS-35; 8, aged CANS-35.

RESULTS

Characteristics of hybrid cell lines and expression of RTV. Hybridomas (HINS lines) between a mouse myeloma cell line, NS1 (P3-NS1/1-Ag4-1), and human monocytes initially showed no macrophage properties as assessed by morphology, cell adherence, surface markers, rosette formation, and phagocytic, chemotactic, and lysozyme-producing functions but gained such properties after prolonged culture (more than 38 weeks). Conversely, κ light-chain-producing ability and PC-1 antigens, found in the hybrids during the early period after cell fusion (8 weeks), were lost from the hybridomas when they expressed macrophage properties. Because hybridomas expressing macrophage properties exhibited mouse but not human antigens, loss of human chromosomes and a repetitive Alu gene family, BLUR8, and expression of mouse macrophage-specific genes, the macrophage proper-

FIG. 2. Structures of MLRV DNA-containing EMBL3 clones. (a) Clone HN15; (b) clone C4B; (c) clone HN7; (d) overall structure of an MLRV DNA-containing locus suggested by the structures of clones HN15, C4B, and HN7; (e) clone C4D. Abbreviations for restriction sites: X, *Xba*I; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; V, *Pvu*II; Sm, *Sma*I; K, *Kpn*I; S, *Sal*I. Symbols: □, viral LTR sequences; ■, N-myc exons 1, 2, and 3; ——, location of probe E-B (*Eco*RI-*Bam*HI fragment), P-P (*Pst*I-*Pst*I fragment), or D (deletion fragment obtained by using exonuclease III and mung bean nuclease). Arrows indicate the direction and extent of sequencing. (f) Nucleotide sequence of the MLRV and surrounding N-*myc* exon 3 sequences in C4B and HN7. Amino acid sequences of N-*myc* exon 3 are shown above the DNA sequence. The large boxed region shows the MLRV sequence. Base mismatches relative to MoMuLV (2, 48) are indicated under the sequence. Symbols: -, gap; ▼, restriction enzyme cleavage site; *, stop codon; ●, nucleotide change resulting in an amino acid difference. Direct repeats are boxed; inverted repeats are overlined; the putative polyadenylation signal is underlined.



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 Q  H  N  Y  A  A  P  S  P  Y  V  E  S  E  D  A  P  P  Q  K  K  I  K  S  E  A  S  P  R
CAGCACAACTATGCTGCACCCTCACCCTACGTGGAGAGCGAGGACGCGCCCCCGCAGAAAAAGATCAAGAGCGAGGCTTCTCCACGC
 P  L  K  S  V  V  P  A  K  A  K  S  L  S  P  R  N  S  D  S  E  D  S  E  R  R  R  N  H
CCCCTCAAAAGTGTTGTTCCAGCAAAAGCGAAGAGCCTGAGCCCCCGAAACTCAGACTCGGAGGACAGCGAGCGCCGCCGCAACCAC
 N  I  L  E  R  Q  R  R  N  D  L  R  S  S  F  L  T  L  R  D  H  V  P  E  L  V  K  N  E
AACATCCTGGAGCGTCAGCGCCGGAACGACCTGCGCTCCAGCTTCCTGACGCTCAGGGACCATGTGCCTGAGCTGGTGAAGAACGAG
 K  A  A  K  V  V  I  L  K  K  A  T  E  Y  V  H  A  L  Q  A  N  E  H  Q  L  L  L  E  K
AAGGCCGCCAAGGTGGTCATCTTGAAAAAGGCCACCGAGTACGTGCACGCCCTACAGGCCAACGAGCACCAGCTCCTGCTGGAAAAG
       Pst I          ←N-myc exon 3, 5'—5'LTR→
 E  K  L  Q  A  R  Q  Q  Q  L  L  K  K  I      IR
GAGAAACTGCAGGCGAGGCAGCAGCAGTTGCTAAAGAAGATCTGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGC
                                    ***                                  Pvu II
CATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTCAGGAACAGAAA-AACAGCTGAATATGGGC
                                                                        TGG
CAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGT
 Xba I
TTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTT
CTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGCGCGCCAGTCCTCCGATTGACT
       Sma I Kpn I                                                      G
GAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTC
                  ←5'LTR—
                    IR
TGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATCGGGAGACCCCTGCCCAGGGACCACCGACCCA
CCACCGGGAGGTAAGCTGGCCAGCAA----------------------------gag, pol, env (6.1 kb)------------
----------------------TCATCACAGTAAACAACAATCTCACCTCTGACCGGGCTGTCCAGGTATGCAAAGATAATAAGTG
                                                          A
GTGCAACCCCTTAGTTATTCGGTTTACAGACGCCGGGAGACGGGTTACTTCCTGGACCACAGGACATTACTGGGGCTTACGTTTGTA
                          Bam HI
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                                                  G
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                                                  A                  Kpn I
TGACAAAACCCAAGAGTGCTGGTTGTGTCTAGTAGCGGGACCCCCCTACTACGAAGGGGTTGCCGTCCTGGGTACCTACTCCAACCA
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                                                                     Kpn I
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      G                           Bam HI
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                                                  A
CCTGGCCCTATTATTGGGTGGACTAACCATGGGGGGAATTGCCGCTGGAATAGGAACAGGGACTACTGCTCTAATGGCCACTCAGCA
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CTCATTGTACTCCTAATGATTTTGCTCTTCGGACCCTGCATTCTTAATCGATTAGTCCAATTTGTTAAAGACAGGATATCAGTGGTC
                          Pvu II
CAGGCTCTAGTTTTGACTCAACAATATCACCAGCTGAAGCCTATAGAGTACGAGCCATAGATAAAATAAAAGATTTTATTTAGTCTC
                ←3'LTR—
                  IR
CAGAAAAAGGGGGGAATGAAAGACCCC----------U3, R (420 bp)---------CAGTTGCATCCGACTTGTGGTCTC
                                    ←3'LTR—                  —N-myc exon 3, 3'→
                                      IR                    E  H  A  R  T  C
GCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCAGATCGAACACGCTCGGACTTGCTAAACG
TTTCCCACACGGACAGTCACTGCCACTTTGCACATTTTGATTTTTTTTTTTTTTAAACAAACATTGTGTTGACATTAAGAATGTTGG
TTTACTTTCAAATTGGTCCCCTGTCGAGTCTGGATCTGGGTAGGGGGCAGGAC
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TABLE 1. Reverse transcriptase activity in cell culture supernatant

Cells	Reverse transcriptase activity (cpm/ml, 10^4)
Early HINS-B3 (8 wk)	0.31
Aged HINS-B3 (38 wk)	0.40
Early CANS-20 (8 wk)	0.33
Aged CANS-20 (20 wk)	0.32
NS1	0.31
JLS-V9	6.43

ties could be attributed to NS1 genes (44). By using mRNA from one of these cell lines, HINS-B3, before and after the expression of macrophage properties, an aged-early HINS cell subtracted cDNA library was constructed and selected for aged HINS-specific cDNA clones. It was found that the library contained a large group of cDNAs related to RTV, together with mouse ferritin heavy-subunit cDNA, reaching 60% of aged HINS-specific clones (31). A ^{32}P-labeled HIC4 probe representing the RTV group clearly demonstrated that with Northern blot analysis, transcription of RTV was greatly enhanced in aged HINS lines (Fig. 1). Similar results were observed in five different HINS lines. Enhanced expression of RTV was not observed in parental NS1 cells or macrophages (data not shown). We also established a range of intraspecific hybrid lines between mouse macrophages and the NS1 line (CANS lines) (57) and found that aged cells of these lines showed similar properties (Fig. 1).

Characteristics of RTV. Clone HIC4 had a 3′ long-terminal-repeat (LTR) sequence similar to that of MoMuLV, with a short 3′ *env* gene sequence; the sequence indicated a few deletions and substitutions of nucleotides (data not shown). To define the structure of the proviral and flanking sequences, we constructed an EMBL3 genomic library and screened it with a HIC4 probe. A single screening yielded several clones associated with the probe. Restriction enzyme map profiles suggested that an MLRV sequence was inserted into at least two sites. Clones C4B, HN7, and HN15, representing one type, and clone C4D, representing the other type, are depicted in Fig. 2. MLRV sequences in both types had the same restriction sites and seemed to have identical proviral sequences. Restriction sites of these clones corresponding to the *env* sequence showed a difference between those of MLRV and proviral MoMuLV sequences. For example, MLRV had two *Bam*HI sites in the *env* region, whereas proviral MoMuLV had one. To observe these differences more directly, several regions corresponding to the proviral sequence of clones C4B and HN7 were sequenced (Fig. 2b and c). The overall structure was very similar to that of proviral MoMuLV, although several sequences were different (2, 45) (Fig. 2f). For example, a single substitution of G for A adjacent to the 5′ side of the *Mbo*I site in the *env* sequence generated the second *Bam*HI site. The three single-nucleotide changes in the *env* sequence resulted in amino acid differences (Fig. 2f). Although MLRV is considered to be closely related to MoMuLV, enhanced production of mature virus has not been detected in the culture medium of aged hybridomas when assessed by reverse transcriptase activity (Table 1).

Expression of N-*myc* by insertion of RTV. MLRV is believed to be transcribed, reverse transcribed, and then reintegrated into the genome. Insertion of the MLRV sequence should cause expression of neighboring sequences. To test this possibility, we performed Northern blot analysis of RNA from HINS and CANS cells, using an *Eco*RI-*Bam*HI (E-B) sequence as a probe (Fig. 2b). The probe detected 2.1-kb transcripts in aged but not early HINS and CANS cells (Fig. 3a). When these experiments were performed with RNA from each of three other HINS and CANS lines, similar results were observed. Next, we sequenced several regions of the E-B sequence and found that it contained the mouse N-*myc* exon 1 5′ and 5′-flanking sequences (11, 23, 52). We further analyzed regions between the E-B and the 5′ LTR sequences of clone C4B (Fig. 2b) and downstream of the 3′ LTR sequence of clone HN7 (Fig. 2c). Restriction enzyme profiles and partial sequencing analysis showed that the former region consisted of the 3′ region of exon 1 following the E-B sequence, the first intron, exon 2, the second intron, and the 5′ region of exon 3 of the mouse N-*myc* genomic sequence. However, the latter consisted of the exon 3 3′ and 3′-flanking regions. Figure 2f also shows the junction sites, including upstream and downstream regions, and clearly demonstrates that these sequences have several features characteristic of other transposable elements (13, 30, 47, 53). First, the MLRV sequence ends in a terminal inverted repeat. Second, the 5′ inverted repeat begins with TG, and the 3′ inverted repeat ends with CA. Third, each end of the MLRV LTRs is flanked by a direct repeat (GATC). The sequence of the junction sites also indicates that the sequence of the 5′-flanking region matches that of mouse N-*myc* exon 3 (Fig. 2c) and that the first three nucleotides, TGA of the 5′ LTR, form a stop codon, predicting termination of protein synthesis and production of a C-terminal split N-*myc* protein. Southern blot analysis showed that a *Pst*I-*Pst*I (P-P) sequence probe (Fig. 2c) detected 7.9-kb *Bam*HI and 7.1-kb *Xba*I rearrangements specific to aged HINS and CANS cells, reflecting the predicted structure of the N-*myc* locus in aged cells (Fig. 2 and 4). These results demonstrate that RTV insertions take place in a narrow region of N-*myc* sequence. Northern blot analysis with the P-P probe (Fig. 2c) also detected 2.1-kb transcripts in all RNA from aged HINS and CANS cells (Fig. 3b), suggesting that integration of RTV into DNA in each cell line must have occurred at the same nucleotide position or in the region nearest to the insertion site in HINS-B3 cells. In contrast, a D probe containing the N-*myc* exon 3 3′ region detected no transcripts (Fig. 3c). The P-P probe hybridized with 2.8-kb mRNA from murine neuroblastoma lines, but autoradiographic intensities in these cell lines were less marked than those in aged HINS and CANS cell lines (Fig. 3d). N-*myc* exons 1 and 2 and the 5′ region of exon 3 approximately predicted the size of the 1.66-kb transcript, indicating that it was 0.44 kb shorter than the transcript in the Northern blot. Therefore, the 3′ untranslated region of the truncated N-*myc* mRNA must be formed with the 5′ LTR that lacks tandem repeat sequences. These results indicate production of a truncated N-*myc* mRNA but not an N-*myc*–virus fusion protein. We have recently cloned and sequenced an N-*myc*-related cDNA clone containing complete coding and 3′ untranslated regions, and the results confirmed these predictions (to be published).

DISCUSSION

The results presented here show that enhanced transcription of endogenous RTV occurs in aged HINS and CANS lines. Restriction enzyme and nucleotide sequencing analyses reveal that the RTV has characteristics closely similar to those of integrated MoMuLV. Parental cells of these cell

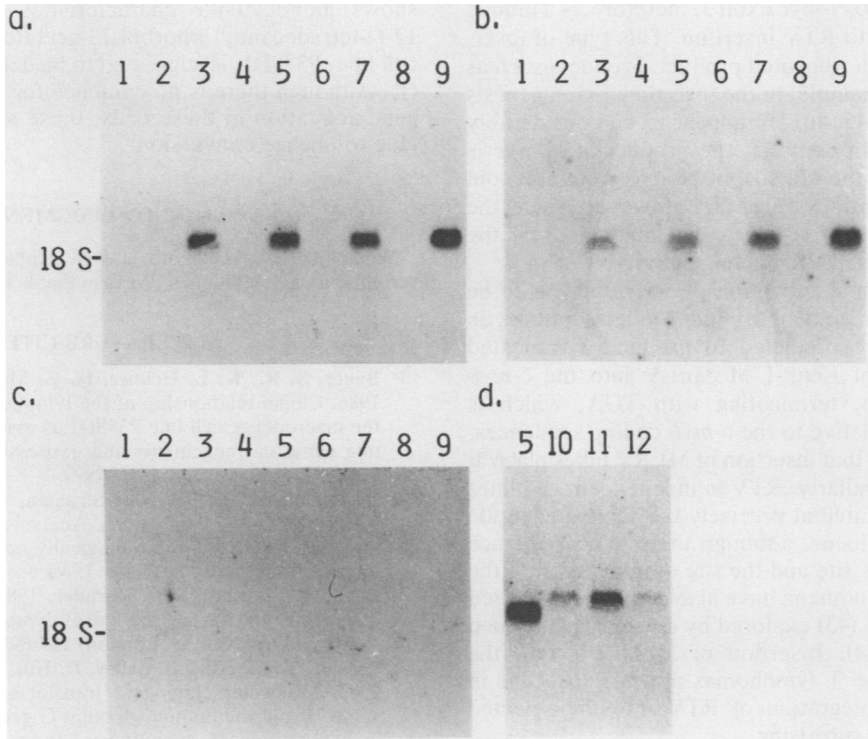

FIG. 3. Expression of cellular sequences flanking the integration site of the MLRV sequence. Total RNA (30 μg per lane) was electrophoresed in a 1.5% formaldehyde gel, blotted on a nylon membrane, and hybridized with ^{32}P-labeled probe E-B (a), P-P (b), D (c), or P-P (d). Lanes contained RNA from the following sources: 1, NS1; 2, early HINS-A4; 3, aged HINS-A4; 4, early HINS-B3; 5, aged HINS-B3; 6, early CANS-20; 7, aged CANS-20; 8, early CANS-35; 9, aged CANS-35; 10, C1300; 11, N18; 12, N20.

lines, NS1, and macrophages have no history of exogenous ecotropic MoMuLV infection, and the cultures cannot be inadvertently infected with MoMuLV because our laboratory has no sources of the virus. We therefore believe that MLRV must have arisen from endogenous sequences in NS1, although there have been no reports of this type of RTV (7, 28). Precise sequencing analysis of MLRV will enable us to examine interrelationships between MLRV and MoMuLV. Reverse transcriptase analysis shows that MLRV fails to produce a mature virus. This may in part be attributable to point mutations in the *env* sequence. In this context, it has been reported that proviral MoMuLV carrying defects in the *gag-pol* regions of the proviral genome in Mov substrains fails to produce infectious virus (39).

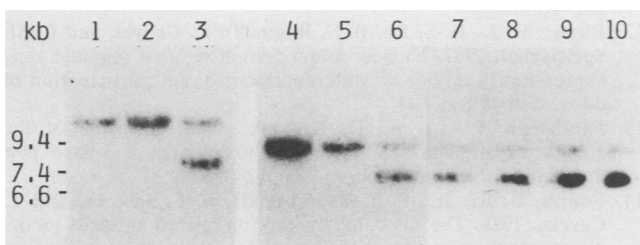

FIG. 4. Southern blot analysis of genomic DNA. Genomic DNA samples were extracted from cell lines, digested with *Bam*HI (lanes 1 to 3) and *Xba*I (lanes 4 to 10), electrophoresed on 1% agarose gels, and blotted onto nylon membranes. The membranes were hybridized with a ^{32}P-labeled P-P probe. Lanes contained DNA from the following sources: 1, NS1; 2, early HINS-B3; 3, aged HINS-B3; 4, NS1; 5, early HINS-B3; 6, aged HINS-B3; 7, aged HINS-A4; 8, aged HINS-A5; 9, aged CANS-20; 10, aged CANS-35.

Enhanced expression of N-*myc* by gene amplification frequently manifested as homogeneously stained regions and double-minute chromosomes has been studied extensively (40, 42). However, it has recently been found that N-*myc* is frequently activated by insertion of MoMuLV in MoMuLV-induced murine T lymphomas. Our experiments also show that N-*myc* is spontaneously transcribed in aged HINS and CANS cells by transposition of MLRV into the N-*myc* locus. The integrated RTV in our cell lines is oriented in the same transcriptional direction as N-*myc*, and N-*myc* positioned upstream of RTV is expressed, which suggests expression governed by an enhancer. Similar findings were also observed for MoMuLV-induced T lymphomas. It is noted that such mechanisms induce higher levels of expression of N-*myc* in aged HINS and CANS cell lines than in murine neuroblastoma cell lines. These findings, therefore, show that RTV involvement in activation of N-*myc* is not a rare event and provides a useful model for analysis of activation and function of N-*myc*.

Several forms of insertional activation by RTV and other movable elements such as LINE (long interspersed repetitive sequence) and intracisternal A particles have been reported (6, 8, 24, 32). There are many reports of insertion near a cellular oncogene locus and, to a lesser extent, into an oncogene locus. However, activation by insertion within the coding region is limited (6, 34). Sites of insertion of MoMuLV into N-*myc* in murine T lymphomas occur in downstream regions of the coding area, although they appear to be near these areas in aged HINS and CANS cells.

Insertion of MLRV into N-*myc* exon 3, therefore, is a unique situation with respect to RTV insertion. This type of insertion seems to produce a truncated product. Products such as those described, for example, in the insertional mutagenesis of *erbB* and *myb* (33, 45, 46, 56) appear to be considerably shorter than normal. In contrast, the product of N-*myc* is predicted to be nearly the full size of N-*myc* protein devoid of C-terminal hexapeptides (Fig. 2f). However, since the leucine zipper region (29) seems to be intact, loss of the peptides may not affect DNA-binding activity.

Findings for insertional activation of c-*myb* appear to be very similar to our results (56): independent monocytic leukemia cell lines are predicted to produce a truncated mRNA by insertion of Leuk-1 MoMuLV into the c-*myb* exon 6 coding region, terminating with TGA, which is introduced in frame relative to the c-*myb* coding sequences. Our experiments show that insertion of MLRV takes place at a few specific sites. Similarly, RTV in independent cell lines are shown to be integrated at precisely the same nucleotide position in the c-*myb* locus, although there is no sequence homology between this site and the site of integration of the N-*myc* gene. Such phenomena have also been demonstrated for the *pim*-1 oncogene (43) explored by an approach termed transposon tagging (34). Insertion of MoMuLV into the N-*myc* locus in murine T lymphomas appears to occur in very limited areas. Integration of RTV into the selected sites, therefore, is not surprising.

It must be stressed that insertional activation of N-*myc* exon 3 by MLRV is observed in all aged HINS and CANS cells. Parental NS1 cells are reported to have rearranged c-*myc* and c-*mos* and genetic elements capable of transforming mouse fibroblasts in transfection systems, and they are considered to maintain autonomic proliferation and immortality (4, 8, 9, 35, 38). N-*myc* transcripts, therefore, may at least in part substitute for the function of transcripts of other oncogenes. We have also shown that aged HINS and CANS cells show a constitutive enhancement of c-*fos* expression, although this phenomenon is not linked to RTV insertion and may result from another process (20). Since both of these nuclear proteins, N-*myc* and c-*fos*, are expressed in limited developmental stages, it is also possible that these genes play a part in cell differentiation: expression of these oncogenes, which may exert their effects by altering gene expression (12, 14), may interfere with the programming of committed NS1 genes and cause switching to a macrophage lineage.

There have been several observations of oncogenes that serve as references. Two cell lines transformed by *ras*-containing viruses differentiate into cells with phenotypic and functional characteristics of mature macrophages (21). B lymphocytes from Eμ-*myc* transgenic mice frequently change into macrophages when infected with v-*raf*-containing viruses (25). Transposable activation of c-*myb* by RTV is found in mouse monocytic cell lines (17, 45, 46). A single point mutation in the v-*ets* oncogene has been reported to affect both erythroid and myeloid cell differentiation (16). These reports suggest that an oncogene alone or in combination with other oncogenes may play specific roles in macrophage cell lines. Several reports have also suggested that B-lineage cell lines can be induced to express macrophage functions. An Abelson virus-induced pre-B-lymphoma cell line changes into macrophagelike cells after exposure to the demethylating drug 5-azacytidine (3). A human B-lymphoid cell line derived from acute leukemia shows monocytelike characteristics after treatment with 12-*O*-tetradecanoyl phorbol-13-acetate (51). A macrophage cell line, P388D1, is suggested to be derived from a B lineage (1). Although there is no evidence for involvement of oncogene activation in these cells, these studies may provide a clue to lineage conversion.

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