JOURNAL OF VIROLOGY, Jan. 1993, p. 112–118
0022-538X/93/010112-07$02.00/0

Vol. 67, No. 1

Mouse Mammary Tumor Virus with Rearranged Long Terminal Repeats Causes Murine Lymphomas

SHIN-ICHI YANAGAWA,[1] KAZUHIRO KAKIMI,[2] HARUTAKA TANAKA,[1] AKIRA MURAKAMI,[1] YOSHIAKI NAKAGAWA,[1] YOSHINAO KUBO,[1] YOSHIHIRO YAMADA,[3] HIROSHI HIAI,[3] KAGEMASA KURIBAYASHI,[4] TORU MASUDA,[4] AND AKINORI ISHIMOTO[1]*

Department of Viral Oncology, Institute for Virus Research,[1] and First Division of Internal Medicine,[2] First Division, Department of Pathology,[3] and Institute for Immunology,[4] Faculty of Medicine, Kyoto University, Kyoto 606, Japan

Received 10 March 1992/Accepted 4 October 1992

Mouse mammary tumor virus (MMTV) is a slowly transforming retrovirus associated primarily with the induction of mammary tumors. It is widely accepted that T-cell lymphomas of various mouse strains are associated with extra proviruses of MMTV. These extra proviruses showed site-specific rearrangements in the U3 region of long terminal repeats (LTRs), consisting of about 400 nucleotide deletions and occasional substitution resulting in unique tandem repeats. However, the question of whether these mutant MMTVs cause lymphomas has not been experimentally resolved. Here we present distinct evidence that they do. We constructed chimeric MMTVs by replacing the LTR of the recently constructed pathogenic MMTV provirus clone with rearranged LTRs of MMTV proviruses obtained from two DBA/2 mouse lymphoma cell lines, MLA and DL-8, and inoculated them into BALB/c mice. These mice developed lymphomas, but no mammary tumors, 4 to 11 months postinoculation, whereas the original pathogenic MMTV clone alone induced mammary tumors. These results showed that the tissue specificity of MMTV tumorigenesis is determined by the LTR structures.

Mouse mammary tumor virus (MMTV) is associated primarily with induction of mammary adenocarcinoma (23). However, there is extensive literature reporting the association of MMTVs with both normal lymphoid cells and lymphoid tumors. As early as 1964, Stuck et al. described the ML (mammary leukemia) antigen of DBA/2 mouse lymphoid leukemias which was shared by MMTV-producing mammary tumors (38). A number of classical electron microscopic reports have also visualized the occurrence of intracytoplasmic A particles, the pronucleocapsids of MMTV (39), in various mouse lymphomas (8, 41, 42). More recently, several investigators have reported amplification of newly acquired MMTV provirus copies in T-cell lymphomas in GR (24–26), DBA/2 (18, 45), BALB/c (11, 13), and C57BL/6 (11, 13, 17) mice; most of these extra proviruses have site-specific rearrangements in the U3 region of their long terminal repeats (LTRs). In particular, all spontaneous T lymphomas of GR and DBA/2 mice so far examined have variant MMTVs, the rearranged LTRs of which exhibit much higher transcriptional activities in T-cell lines than do the wild-type LTRs (40, 45). We previously reported that rearranged LTRs from two DBA/2 mouse lymphomas, MLA and DL-8, not only lacked the negative regulatory element of transcription through deletion of specific sequences but also acquired a novel enhancer element by specific point mutations, and that both of these rearrangements substantially contributed to the higher transcriptional activities of these LTRs in T cells (45). These observations suggest that MMTV variants are involved in the induction of the lymphomas. However, most of the lymphomas fail to produce infectious virions (29, 32, 42); consequently, the leukemogenicity of these variant MMTVs has not been directly demonstrated. The only exception is DMBA-LV, which is a thymotropic type B retrovirus isolated from carcinogen-induced thymomas of CFW/D mice (5). This virus induces thymomas, but not mammary tumors, when inoculated intrathymically into newborn mice (2), and its LTR has a typical rearrangement (4). However, the proviral structure other than the LTR was not fully analyzed, and the pathogenic provirus clone was not available, leaving the possibility that variation in the structural genes also contributes to the leukemogenicity of this virus. In carcinogen-induced lymphomas of C57BL/6, BALB/c, and RF/J (16) mice with variant MMTV proviruses, however, it is very likely that amplified proviruses are simply passenger viruses of either exogenous or endogenous origin, adapted to lymphoid cells through LTR rearrangements, but are not actual etiological agents of these lymphomas. Therefore, whether MMTVs are the actual causative agents in the induction of certain T-cell lymphomas with amplified proviruses is still open to debate. Recently, Shackleford and Varmus (34) have surmounted the difficulties of cloning MMTV proviruses (7, 20) and have constructed a pathogenic MMTV provirus clone, hybrid MMTV, which enabled us to address the issues discussed above by using a molecular genetic approach.

MATERIALS AND METHODS

Construction of chimeric MMTV proviruses and establishment of MMTV-producing cells. A pathogenic MMTV provirus clone, hybrid MMTV, was provided by H. E. Varmus (34). Two T-cell lines, MLA and DL-8 (45), were established from spontaneous lymphomas of a DBA/2 mouse in our laboratory. In a previous report (45), MLA was classified as a B-cell line, but cell surface marker analysis revealed that it is in fact a T-cell line (Thy1.2$^+$ CD4$^+$ CD8$^+$ CD3$^+$ J11D$^+$). Rearranged MMTV LTRs from MLA and DL-8 cell lines were designated MLA LTR and DL-8 LTR, respectively; both LTRs have been previously characterized (45). *Pst*I-

* Corresponding author.

*Sac*I fragments of 0.7 kb from MLA and DL-8 LTRs were isolated from MLA-1 PUC and DL-8 PUC (45), respectively. MLA MMTV and DL-8 MMTV were constructed by replacing the 1.1-kb *Pst*I-*Sac*I region of the 3′ LTR of hybrid MMTV plasmid with the corresponding region of the variant LTRs described above. Rat XC cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum. XC cells were cotransfected with 30 μg of MMTV provirus plasmid and 1 μg of plasmid PSV2neo (35) by using calcium phosphate (21) and were selected with G418 (Geneticin; GIBCO) at 400 μg/ml. The G418-resistant clones were treated with dexamethasone (1 μM) for 48 h to stimulate virus expression, and those expressing MMTV RNAs were further screened by Northern (RNA) blotting. To roughly normalize the virus titers for inoculation, clones expressing similar amounts of viral RNAs were collected. These virus-producing XC cell clones were used for animal infection.

Animal infection. Four-week-old BALB/c mice were infected with MMTVs by injection with virus-producing cells as described by Shackleford and Varmus (34). Cells (10^7) grown in the presence of 1 μM dexamethasone for 48 h were suspended in 1 ml of phosphate-buffered saline, and then 0.5 ml of cell suspension was injected into each mouse subcutaneously and intraperitoneally. Infected mice were checked three times per week for tumor development.

Southern blotting analyses. High-molecular-weight DNAs from tumors were isolated by standard procedures (21). Twenty micrograms of genomic DNA was digested with restriction enzymes, separated on 0.8% agarose gels, and then Southern blotted (21). Probe labeling and hybridization were performed as previously described (44).

PCR. Sense and antisense primers (20 bases long) used for LTR sequence amplification corresponded to positions 18 to 38 and 1296 to 1315, respectively, of the C3H MMTV LTR (Fig. 1A). Amplification was performed in 100 μl of reaction mixture consisting of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% (wt/vol) gelatin, 1.5 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate, 1 μM each sense and antisense primers, 2.5 U of *Taq* polymerase (Perkin Elmer-Cetus) and 1 μg of genomic DNA from each lymphoma. Amplification proceeded under standard conditions (annealing, primer extension, and denaturation steps were at 53°C for 30 s, 72°C for 90 s, and 94°C for 30 s, respectively) for 40 cycles with a Perkin Elmer-Cetus thermal cycler. Polymerase chain reaction (PCR) reaction products were applied to a 1.5% agarose gel, and the bands corresponding to the novel LTRs were recovered. DNAs were extracted from the agarose and cloned into the *Hin*cII site of pUC119, using standard procedures.

RESULTS

Induction of lymphomas by chimeric MMTV clones. Using the hybrid MMTV clone, we prepared two chimeric MMTVs by replacing the U3 region of the 3′ LTR with the corresponding regions of MLA and DL-8 MMTV LTRs (45). After one cycle of virus replication, the 3′ LTR structure was found in the 5′ LTR of the newly generated viruses (Fig. 1B). Rat XC cells were transfected with these MMTV proviruses, resulting in the establishment of cell lines producing these chimeric MMTVs. Thereafter, 4-week-old BALB/c mice of both sexes were inoculated with 5×10^6 virus-producing cells subcutaneously and intraperitoneally, and tumor development was observed. Table 1 shows the tumor incidence among these mice 12 months postinoculation. In accordance with results reported by Shackleford and Varmus (34), 57% of female mice inoculated with the original hybrid MMTV clone developed mammary tumors but no lymphomas. In sharp contrast, mice inoculated with chimeric MLA MMTV and DL-8 MMTV developed lymphomas at incidences of 29 and 20%, respectively, irrespective

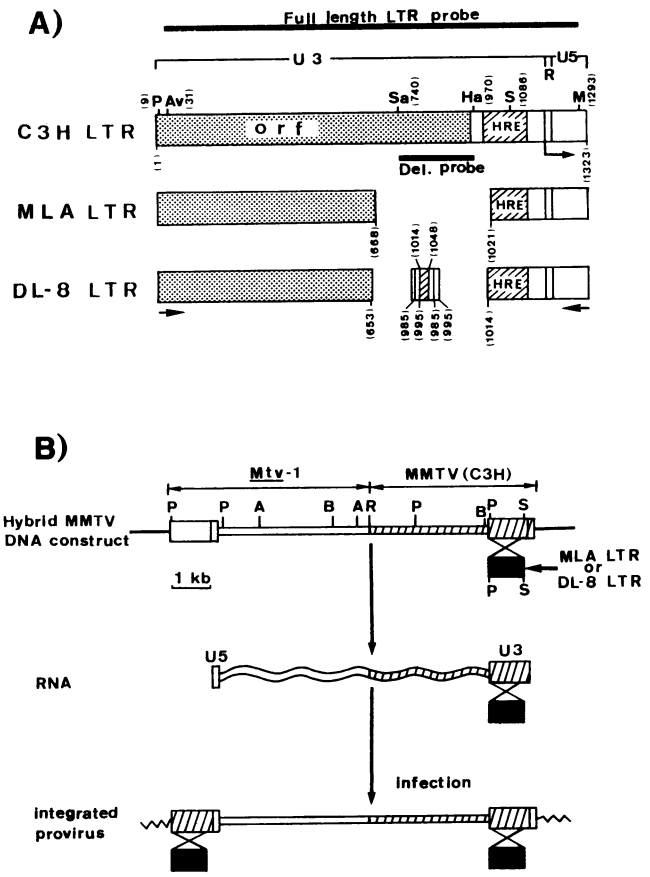

FIG. 1. Structures of LTRs and provirus constructs used in this study. (A) Schematic representation of the structures of the milk-borne MMTV LTR from C3H mice and the rearranged MMTV LTRs from the mouse T-lymphoma cell lines MLA and DL-8. The structures and transcriptional activities of MLA and DL-8 LTRs have been previously described in detail (38). The general organization of wild-type MMTV LTR is shown at the top. The hormone-responsive element (HRE), LTR ORF, and transcription initiation site (arrow) are indicated. Numbers refer to positions in the C3H LTR (17). The deletions in the rearranged LTRs are indicated by interrupted boxes. The reinserted sequences and positions of these sequences in the C3H LTR are also shown in DL-8 LTR. By rearrangement, ORFs in MLA and DL-8 LTRs become 228 and 233 codons, respectively, compared with the 319 codons in the C3H LTR. The ORF proteins encoded by both rearranged LTRs have truncated carboxy-terminal ends. Two arrows under the DL-8 LTR show positions of primers used for PCR. (B) Structures of the hybrid MMTV provirus plasmid and its LTR derivatives, MLA MMTV and DL-8 MMTV plasmids. In the hybrid MMTV provirus plasmid, the 5′ half of the provirus consists of *Mtv-1* DNA (open boxes) and the 3′ half originated from C3H MMTV (hatched boxes). MLA and DL-8 MMTVs were constructed by replacing the U3 region of the 3′ LTR of hybrid MMTV with the corresponding regions of MLA and DL-8 LTRs, respectively (shaded box). Restriction enzyme sites: P, *Pst*I; Av, *Ava*I; S, *Sac*I; M, *Msp*I; R, *Eco*RI; B, *Bgl*II; A, *Acc*I; Sa, *Sau*3AI; Ha, *Hae*III.

TABLE 1. Tumor incidence 12 months postinjection[a]

Virus infected	No. of mice (female, male) infected	No. of mice (female, male) that developed:		Avg latency period (wk)
		Mammary tumor	Lymphoma	
None	54 (24, 30)	0	0	
Hybrid MMTV	44 (21, 23)	12 (12, 0)	0	33
MLA MMTV	24 (11, 13)	0	7 (2, 5)	34
DL-8 MMTV	49 (30, 19)	0	10 (6, 4)	26

[a] Two independent virus-producing clones were tested for each MMTV, and both induced tumors in recipient mice. XC cells containing only the *neo* gene were inoculated into control mice. Mice infected with DL-8 and MLA MMTVs developed lymphomas within 4 to 11 months postinoculation, whereas hybrid MMTV-induced mammary tumors arose within 5 to 10 months postinoculation. Mice were killed when they showed advanced signs of diseases (splenomegaly and lymphadenopathy). Enlarged spleens, peripheral lymph nodes, and thymuses were pooled for biochemical analyses and stored at −80°C. Mammary tumors were similarly processed.

of gender difference, while no mammary tumor was observed. DL-8 MMTV, whose LTR had three- to fivefold higher transcriptional activity in vitro than did the MLA LTR (45), induced lymphomas much sooner in vivo than did MLA MMTV (as judged by the average latency period). No tumors developed in control mice, indicating that XC cells alone have no effect on tumor incidence. Immunocytological analyses with a fluorescence-activated cell sorter showed that most of these lymphomas were immature T cells ($CD3^+$ $CD4^+$ $J11D^+$) or immature lymphoblasts that expressed neither Thy-1, CD3, nor surface immunoglobulin. T-cell receptor (TCR) β-chain rearrangement analysis revealed that these lymphomas are clonal or oligoclonal with respect to the rearrangement pattern of this gene (see Fig. 7). Most of these lymphomas were transplantable into nude mice but not into syngeneic BALB/c mice.

Characterization of extra MMTV proviruses in lymphomas. Further studies disclosed that these lymphomas and mammary tumors developed as monoclonal or oligoclonal growths of cells infected with the respective MMTV clones. First, DNAs of all tumor cells contained a 2.3-kb *gag-pol* fragment which is characteristic of both hybrid and chimeric MMTV clones (Fig. 2A). Many lymphoma cells also had unintegrated linear MMTV DNAs (data not shown). Second, the characteristic LTR lengths of the respective MMTV inoculants were stably maintained in acquired MMTV proviruses of these tumors (Fig. 2B) and transcribed as such (see below). Only DL-8 MMTV-induced lymphomas were exceptional in that 4 of 10 lymphomas exhibit an LTR fragment that was identical to that of the DL-8 MMTV clone, while the other 6 harbored proviruses with novel LTRs, longer than the original DL-8 LTR but shorter than the C3H

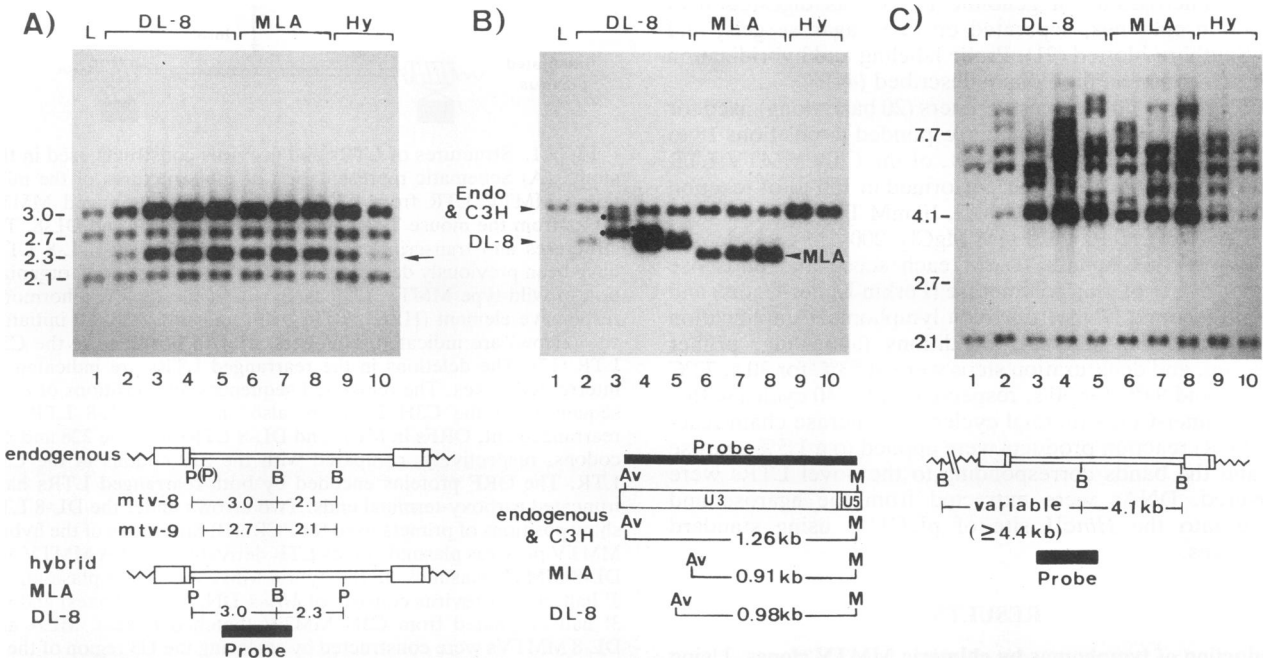

FIG. 2. Characterization of MMTV proviruses in tumors by Southern blotting. (A) Presence of recombinant MMTV proviruses in lymphomas and mammary tumors; (B) characterization of the LTR of the acquired proviruses in each tumor; (C) clonality of the tumor with respect to the integration sites of recombinant MMTV proviruses. High-molecular-weight DNAs from normal BALB/c liver (L; lane 1), DL-8 MMTV-induced lymphomas (lanes 2 to 5), MLA MMTV-induced lymphomas (lanes 6 to 8), and hybrid MMTV-induced mammary tumors (Hy; lanes 9 and 10) were digested with *Pst*I and *Bgl*II (A), with *Ava*I and *Msp*I (B), and with *Bgl*II (C). The DNAs were analyzed by Southern blotting using a *gag-pol* probe (3.1-kb *Acc*I fragment) (A and C) and an LTR probe (1.3-kb *Ava*I-*Msp*I fragment) (B). Positions of the probes and origins of the fragments are schematically represented below the gels. Although BALB/c mice have three endogenous MMTV proviruses (*Mtv-6*, *Mtv-8*, and *Mtv-9*, structures of *Mtv-8* and *Mtv-9* but not *Mtv-6* (consisting of only LTRs) are shown. In panel A, the arrow indicates recombinant MMTV-derived 2.3-kb fragments. In panel B, the positions of wild-type, MLA, and DL-8 LTRs are indicated. The newly generated LTRs in DL-8 MMTV-induced lymphomas are indicated by asterisks. The lymphomas shown in lanes 2 and 5 exhibit the original DL-8 LTR alone. However, two types of novel LTRs were detected in one lymphoma (lane 3), and both novel and DL-8 LTRs were observed in another (lane 4). In panel C, the probe detected a 4.1-kb internal fragment from recombinant MMTVs and the 5′ host-virus junction fragment specific for each acquired provirus in addition to the three fragments derived from endogenous MMTVs.

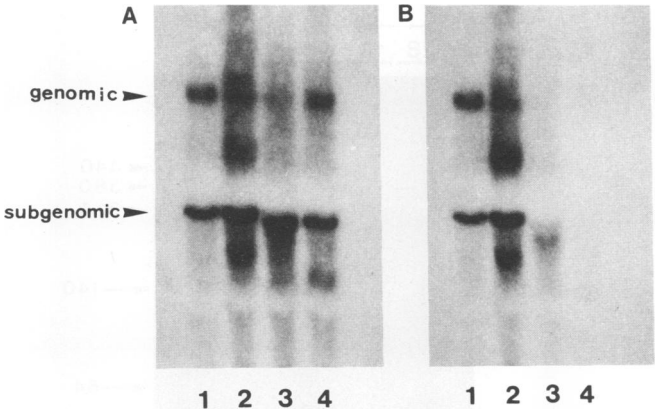

FIG. 3. Expression of recombinant MMTV RNAs in lymphomas and mammary tumors. Total RNAs were isolated by using guanidinium thiocyanate-CsCl (17). Total RNAs (10 μg of each) from C3H MMTV-induced mammary tumor cell line MuMT73 (lane 1), a hybrid MMTV-induced mammary tumor (lane 2), an MLA MMTV-induced lymphoma (lane 3), and a DL-8 MMTV-induced lymphoma (lymphoma number 4; lane 4) were separated on a 1% formaldehyde agarose gel, blotted onto a nylon membrane, and hybridized with a full-length LTR probe as previously described (17, 35) (A). The same membrane was washed and rehybridized with a deletion-specific probe, the 0.23-kb *Sau*3AI-*Hae*III fragment of the C3H LTR (Fig. 1) (B).

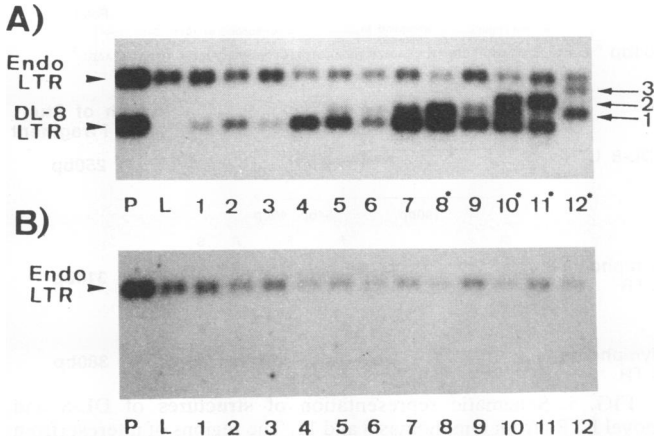

FIG. 4. Further characterization of novel LTRs in DL-8 MMTV-induced lymphomas. DL-8 MMTV plasmid DNA (2 ng; lane P), BALB/c mouse liver DNA (20 μg; lane L), and DNAs from 12 independent lymphomas (20 μg of each; lanes 1 to 12) were digested with *Ava*I and *Msp*I and analyzed by Southern blotting with the full-length LTR probe (A). The same filter was washed and rehybridized with a deletion-specific probe (B). Plasmid DNA was used as the standard for the original DL-8 and wild-type LTRs. Three novel LTRs of different sizes are indicated by arrows 1, 2, and 3. Four lymphomas (lymphomas 8, 10, 11 and 12) from which the DNA was LTR amplified are indicated by asterisks.

LTR. In five of these six lymphomas, however, the original DL-8 LTR was also detected. It is very likely that a tandem repeat structure in DL-8 LTR facilitates further rearrangements of LTRs in vivo, because MLA LTR with a simple deletion remains quite stable. Since the DL-8 virus undergoes LTR changes at a high frequency, the structure of these novel LTRs was analyzed in detail (see below). Third, *Bgl*II-digested DNAs showed a few (2 to about 10) 5′ host-virus junction fragments which were unique for each tumor (Fig. 2C), indicating that each tumor originated from a single or a few cell clones. All lymphomas and mammary tumors expressed MMTV genomic and subgenomic RNAs. However, reflecting LTR rearrangements in MLA and DL-8 MMTVs, the RNA species expressed in lymphomas were shorter than those in mammary tumors (Fig. 3A). Although a faint band of unknown origin was detected in lane 3 of Fig. 3B, a deletion-specific probe failed to detect any hybridizable RNAs in lymphomas, indicating that chimeric MMTVs are preferentially expressed in lymphomas (Fig. 3B). MMTV-related intracytoplasmic A particles were also observed in these lymphomas (data not shown). That MMTVs with mutant LTRs cause lymphomas was further supported by a study using BALB/c-*Fv-4w*^{r} mice (14), which are congenic BALB/c mice that obtained the *Fv-4w*^{r} gene and are thus resistant to infection with various ecotropic murine leukemia viruses. These mice, when inoculated with DL-8 and MLA MMTVs, also developed lymphomas in which these chimeric MMTV proviruses were detected (data not shown), eliminating the possibility that leukemia virus is involved in induction of these lymphomas.

Further characterization of novel LTR structures generated in DL-8 MMTV-induced lymphomas. LTR structures of MMTV proviruses in 12 DL-8 MMTV-induced lymphomas (10 are described in Table 1 and 2 were obtained from the second experiment) were analyzed by Southern blotting performed as described for Fig. 2B. As shown in Fig. 4A, both original DL-8 and novel LTRs were detected in at least seven lymphomas (lymphomas 5 to 11). Two novel LTRs, but not the original, were detected in lymphoma 12. Lymphomas 1 to 4 carried only the original DL-8 LTR. These novel LTRs could be grouped into three classes with respect to their apparent LTR lengths (Fig. 4A). The shortest was detected in seven lymphomas (lymphomas 5 to 10 and 12; indicated by arrow 1 in Fig. 4A). That of median size was present in lymphomas 10 and 11 (indicated by arrow 2), and the longest was present in lymphoma 12 (indicated by arrow 3). When the filter was washed and rehybridized with the deletion-specific probe (Fig. 1A), neither the novel LTRs nor the original DL-8 LTR hybridized with this probe (Fig. 4B), indicating that novel LTRs were not generated by rescuing MMTV LTR sequences that are deleted from DL-8 LTR.

To analyze the structures of novel LTRs more directly, the MMTV LTR sequences in four lymphomas (8, 10, 11, and 12 in Fig. 4A) were amplified by PCR. The profile of the PCR products separated on a 1.5% agarose gel was almost the same as that of the Southern blots shown in Fig. 4A (data not shown). All of the novel LTR sequences thus obtained were subcloned into pUC119 and further analyzed. Among them, two novel LTRs from lymphomas 8 and 11 were sequenced. To exclude possible errors generated by *Taq* polymerase during amplification, the sequences were determined from clones isolated in two separate amplification reactions. The data showed that one and two copies of the 64-bp sequence, the unit of a direct repeat structure of the DL-8 LTR (Fig. 1A and 5), were additionally inserted into the novel LTRs from lymphomas 8 and 11, respectively. Thus, novel LTRs from lymphomas 8 and 11 contain three and four tandemly placed copies of the 64-bp sequence, respectively (Fig. 5). These two novel LTRs are identical in sequence to the DL-8 LTR between regions 5′ to the *Stu*I site and 3′ to the *Sac*I site (Fig. 1A). To determine whether the novel LTRs from different lymphomas of seemingly the same length actually have similar LTR structures, the two

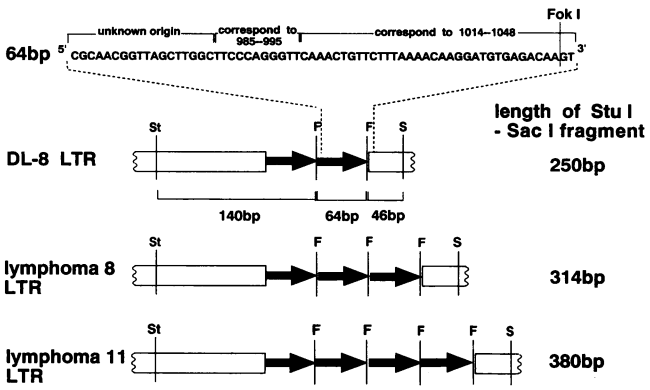

FIG. 5. Schematic representation of structures of DL-8 and novel LTRs from lymphomas 8 and 11. The regions of interest (from the *Stu*I to the *Sac*I sites of the MMTV LTR) are shown. The arrow indicates the 64-bp sequence, the unit of the tandem repeat structure of the DL-8 LTR. Restriction sites: St, *Stu*I; S, *Sac*I; F, *Fok*I. The 64-base sequence is shown at the top. Numbers refer to positions in the C3H LTR sequence.

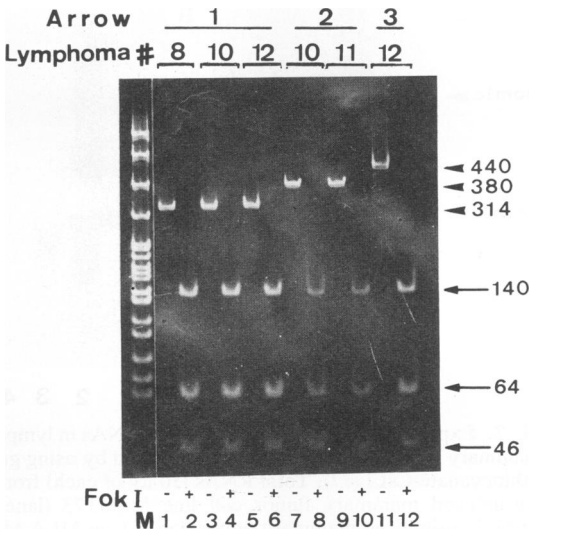

FIG. 6. Structural analysis of the *Stu*I-*Sac*I fragment from novel LTRs. The *Stu*I-*Sac*I fragments from the shortest novel LTRs (indicated by arrow 1 in Fig. 4A) from lymphomas 8, 10, and 12, the mid-size novel LTRs (arrow 2 in Fig. 4A) from lymphomas 10 and 11, and the longest novel LTR (arrow 3 in Fig. 4A) from lymphoma 12 were analyzed on a 5% acrylamide gel before and after *Fok*I digestion. Positions of the 140-, 64-, and 46-bp fragments are indicated. *Msp*I digests of pBR322 were used for determining fragment sizes (lane M).

short LTRs (indicated by arrow 1 in lymphomas 10 and 12 in Fig. 4A), a median-size LTR (indicated by arrow 2 in lymphoma 10), and the longest LTR (indicated by arrow 3 in lymphoma 12) were isolated and digested with *Stu*I and *Sac*I. This analysis revealed that all of these LTRs have sequences of the same size as those in DL-8 LTR with respect to regions 5′ to the *Stu*I site and 3′ to the *Sac*I site (data not shown), indicating that the size difference among these LTRs is a result of their *Stu*I-*Sac*I fragments. Therefore, the *Stu*I-*Sac*I fragments from these six novel LTRs were isolated and analyzed on a 5% polyacrylamide gel. The novel LTRs of the same size were of the same length as the *Stu*I-*Sac*I fragments (compare lanes 1, 3, and 5 and lanes 7 and 9 of Fig. 6). Thus, the novel LTRs indicated by arrows 1, 2, and 3 in Fig. 4A gave about 310-, 380-, and 440-bp *Stu*I-*Sac*I fragments, respectively. If all of these *Stu*I-*Sac*I fragments shown in Fig. 6 contain three to five copies of the 64-bp sequence and are tandemly placed, *Fok*I digestion of these *Stu*I-*Sac*I fragments should generate 140-bp *Stu*I-*Fok*I, 64-bp *Fok*I-*Fok*I, and 46-bp *Fok*I-*Sac*I fragments (Fig. 5). *Fok*I digestion revealed that this was in fact the case (Fig. 6, lanes 2, 4, 6, 8, 10, and 12). These observations suggested that most of the novel LTRs detected in DL-8 MMTV-induced lymphomas were generated by amplification of the 64-bp sequence, the unit of the tandem repeat structure of the DL-8 LTR. Therefore, the LTRs indicated by arrows 1, 2, and 3 in Fig. 4A seemed to contain three, four, and five copies of the 64-bp sequence, respectively.

To confirm that these lymphomas are indeed T cells and to study the clonality of these lymphomas, the rearrangement of the TCR β-chain gene was analyzed. By using the standard protocol, genomic DNAs from lymphomas were digested with *Eco*RI and Southern blotted with the Jβ1 and Jβ2 probes. The Jβ1 probe gave rise to a germ line band of 8.5 kb, while the Jβ2 probe yielded a germ line band of 2.3 kb. In most of the lymphomas, only one or very few bands generated by a specific TCR β-chain gene rearrangement were detected (Fig. 7), indicating that lymphomas are clonal or oligoclonal with respect to the rearrangement pattern of the TCR β-chain gene. Since most of the lymphomas with novel LTRs also carry MMTV proviruses with the original DL-8 LTR and have a single TCR rearrangement pattern, it is very likely that further LTR rearrangements which produced novel LTR structures occurred after clonal establishment of each lymphoma. In other words, a lymphoma clone initially infected with the original DL-8 virus might generate a subpopulation of cells carrying MMTVs with novel LTRs during clonal expansion of lymphoma cells.

DISCUSSION

This study is the first demonstration using cloned infectious MMTV proviruses that specific rearrangement in the U3 region of the LTR can dramatically convert the target tissue of MMTV transformation from mammary epithelial to lymphoid cells. This study with type B retrovirus also provides support for the notion that the main determinants of tissue tropism of retroviruses reside in their LTRs (19, 36). These results indicate that mutant MMTVs are the causative agents of certain mouse lymphomas. For mammary tumor induction, however, MMTV seems to require the DNA sequences that were deleted in the rearranged LTRs or the intact protein product encoded by the LTR open reading frame (ORF), which has recently been identified as an endogenous mouse superantigen (1, 10), or both, because variant MMTVs no longer induce mammary tumors. Participation of ORF proteins in the induction of T-cell lymphomas seems promising in light of recent data on ORF proteins. However, from a comparison of the amino acid sequences of MMTV ORF proteins with different Vβ specificities (10) and from a study of mice transgenic for the wild-type and truncated forms of ORF coding sequences (1), it is widely accepted that the C-terminal 30 amino acids of the ORF coding region are essential for superantigens and could play a crucial role in determination of Vβ specificity. A recent report (15) that identifies the ORF protein as a type II transmembrane glycoprotein (N terminus intracellular and C

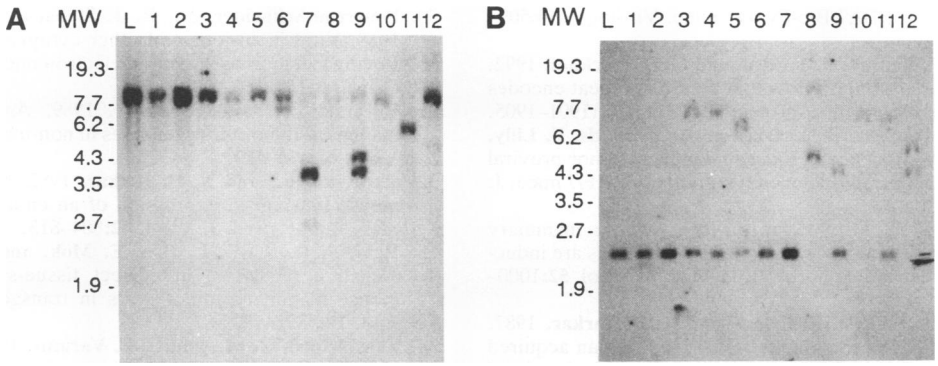

FIG. 7. TCR β-chain gene rearrangements in DL-8 MMTV-induced lymphomas. Twenty micrograms of genomic DNAs from BALB/c mouse liver (L) and 12 lymphomas (lanes 1 to 12) were digested with *Eco*RI and Southern blotted. Probes used were Jβ1 (2.0-kb *Pst*I fragment [22]) (A) and Jβ2 (2.3-kb *Eco*RI fragment [22]) (B).

terminus extracytoplasmic) further supports these observations. The DL-8 and MLA LTRs encode 233 and 228 codons, respectively (Fig. 1), and both LTRs lack about the last 90 carboxy-terminal codons. Therefore, the ORF proteins of these rearranged LTRs probably could not function as superantigens. Direct assay for the function of these truncated ORF proteins is necessary to determine whether these ORFs can stimulate certain T cells. We have not yet analyzed whether variant MMTVs show a drastic shift in tissue-specific expression profile in vivo (e.g., strong lymphotropism or lack of virus expression in mammary glands) compared with the wild-type MMTV. Ross et al. (33) have recently reported a transgenic mouse study in which a rearranged LTR isolated from a C57BL/6 T lymphoma was active in novel tissues, such as the heart, brain, and skeletal muscle, in addition to the regular sites of MMTV expression. An MMTV variant associated with renal adenocarcinoma has a unique LTR rearrangement with a much smaller deletion and a 91-bp foreign DNA insertion (12, 43). MMTV is also associated with a variety of other tumors (31), including Leydig cell tumors (28, 30, 37), mastocytomas (6), ependymoblastomas (3, 27), and pituitary and ovarian tumors (9). It is an attractive idea that these tumors are caused by MMTV variants in which the LTRs are rearranged characteristically. Our system also provides a unique and useful model with which to study the molecular mechanisms involved in the retroviral induction of lymphomas, since BALB/c mice have low susceptibility to cancer, are sensitive to MMTV, and contain only three copies of endogenous MMTV. In addition, the extra proviruses acquired by the lymphomas can be easily identified by their characteristic LTR rearrangements. We screened these T-cell lymphomas for integration of MMTV provirus in the loci of the cellular proto-oncogenes N-*myc*, c-*myc*, *Pim-1*, *Wnt-1*, and *int-2* and of antioncogene P-53 but did not detect any rearrangement in these loci. Thus, we are currently attempting to clarify which proto-oncogene(s) is activated in lymphomas by acquired chimeric MMTVs.

ACKNOWLEDGMENTS

We thank H. E. Varmus for the hybrid MMTV provirus clone and R. Nusse for discussions. We also thank N. Kasai and T. Fukuhara for technical assistance.

This work was supported by grants-in-aid from the Ministry of Education, Science and Culture of Japan to S.-I.Y. and by a grant from the Naito Foundation to S.-I.Y.

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