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An Endogenous Mouse Mammary Tumor Virus Genome Common in Inbred Mouse Strains Is Located on Chromosome 6

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We have examined *Eco*RI-restricted cellular DNA from mouse-hamster somatic cell hybrids. Results of this analysis show that the unit II mouse mammary tumor virus proviral genome is located on mouse chromosome 6. Restriction analysis of cellular DNA from (C3H/OuJ × Czech II) × Czech II backcross mice showed a strong linkage between unit II and *Igk*. The gene order of these markers on chromosome 6 relative to the *Raf* and Kirsten murine sarcoma virus *ras-2* proto-oncogenes was established.

Multiple copies of the mouse mammary tumor virus (MMTV) proviral genome are transmitted in the germ lines of inbred strains of mice (33). The complement of endogenous MMTV genomes varies among different inbred mouse strains. The expression of certain endogenous MMTV genomes is associated with an increased incidence of mammary tumors in several inbred strains. Expression of the endogenous genome at the *Mtv-1* locus is associated with late-occurring tumors in C3Hf and DBAf mice (20, 30). Early, pregnancy-dependent mammary tumors in GR mice are associated with expression at the *Mtv-2* locus (18, 31). Presumably, the MMTV genome expressed in these strains acts as an insertional mutagen in inducing mammary tumors (21).

BALB/c mice, which have a low incidence of spontaneous mammary tumors, carry three endogenous MMTV genomes. These mice express a 1.6-kilobase-pair (kbp) MMTV-related RNA in lactating mammary glands (2, 32, 34). This species of RNA corresponds primarily to the long terminal repeat (LTR) region of the MMTV proviral genome. BALB/c mice are sensitive to the induction of mammary and plasma cell tumors by chemical carcinogens (9, 23). Both types of tumors express the 1.6-kbp MMTV RNA species (2, 32, 34). The proviral genome(s) involved in the expression of this species of RNA has not been identified. The factors which govern the expression of this RNA and its relevance to tumor development are not known.

To answer these questions, we began by determining the chromosomal location of the BALB/c endogenous MMTV genomes. Endogenous MMTV genomes have been classified in different inbred mouse strains primarily on the basis of *Eco*RI restriction enzyme analysis of cellular DNA (3). *Eco*RI cleaves most endogenous MMTV genomes once in the *pol* gene, generating two host-virus junction fragments per provirus. Cosegregating MMTV-related fragments have been designated as units or as *Mtv* loci. The three endogenous MMTV genomes of BALB/c mice are designated units I, II, and III. In a previous report, BALB/c mouse-Chinese hamster somatic cell hybrids were used to locate the proviruses corresponding to units I and III on mouse chromosomes 16 and 12, respectively (4). For localization of the third provirus, we used mouse-Chinese hamster somatic cell hybrids and a sexual cross involving an endogenous MMTV-negative feral mouse strain.

The unit II MMTV proviral genome is defined by 8.3-kbp LTR-*gag-pol*-related and 6.6-kbp *pol-env*-LTR-related

TABLE 1. Correlation between specific mouse chromosomes and unit II in mouse-Chinese hamster somatic cell hybrids[a]

Mouse chromosome	No. of hybrid clones retaining unit II or chromosome				No. discordant/ no. hybrids (%)
	Both	Neither	Unit II only	Chromosome only	
1	6	10	4	5	9/25 (36)
2	8	9	2	7	9/26 (35)
3	5	10	4	1	5/20 (25)
4	6	12	5	3	8/26 (31)
5	1	14	11	1	12/27 (44)
6	10	13	0	1	1/24 (4)[b]
7	12	4	0	11	11/27 (41)
8	5	14	6	1	7/26 (27)
9	7	11	5	3	8/26 (31)
10	4	15	8	1	9/28 (32)
11	0	14	12	0	12/26 (46)
12	5	3	4	7	11/19 (58)
13	7	9	2	1	3/19 (16)
14	5	14	6	2	8/27 (30)
15	8	2	1	8	9/19 (47)
16	7	12	2	2	4/23 (17)
17	9	7	1	7	8/24 (33)
18	6	11	3	0	3/20 (15)
19	7	10	3	3	6/23 (26)
X	8	12	3	1	4/24 (17)

[a] 19 hybrids were karyotyped, and 9 were typed for specific mouse isozyme markers.

[b] The single discordancy lacked unit II but contained mouse *Tpi-1*. However, this hybrid was not karyotyped to confirm the presence of an intact chromosome 6. Characterization of somatic cell hybrids has been described previously (15).

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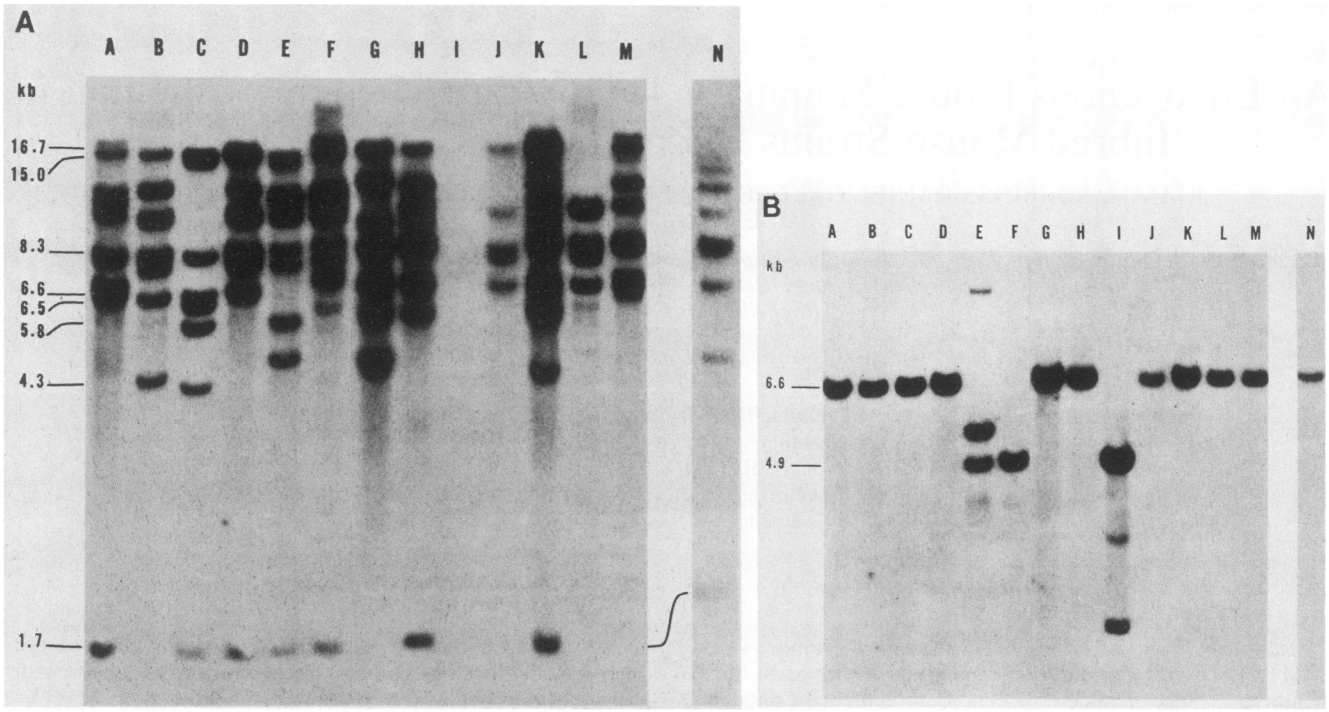

FIG. 1. (A) MMTV-related sequences in *Eco*RI-digested cellular DNA from inbred mouse strains. Lanes: A, GR/Imr; B, AKR/N; C, C3H/OuJ; D, CBA/J; E, CE/J; F, NZB/BlNJ; G, A/J; H, NFS/N; I, *M. musculus musculus* (strain Czech II); J, BALB/cPt; K, DBA/2N; L, C57BL/6N; M, STS/A; N, RIIIS/J. Liver DNAs were prepared as previously described (11). DNA samples (10 μg) were restricted with *Eco*RI by the directions of the manufacturer (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Restricted DNAs were electrophoresed through a 0.7% horizontal agarose gel and transferred by a modification of the method of Southern (27) to nylon membranes. Blots were hybridized with 2×10^6 cpm of ^{32}P-labeled MMTV proviral DNA per ml, prepared by nick translation of a 1.4-kbp *Pst*I fragment of a recombinant clone of the MMTV (C3H) LTR kindly provided by J. Majors and H. Varmus. Hybridization was for 24 h at 37°C in 5× Denhardt solution–3× SSPE (1× SSPE is 180 mM NaCl plus 10 mM NaH_2PO_4, pH 7.4)–1 mM EDTA (pH 7.4)–2.5% dextran sulfate–30% formamide. Washing was done in 0.3× SSC (1× SSC is 0.15 M NaCl plus 0.15 M sodium citrate)–0.05% sodium dodecyl sulfate three times for 20 min each at 65°C. Autoradiography was for 1 to 4 days with two screens at −70°C. (B) Unique unit II 3′ flanking sequence in *Eco*RI-digested cellular DNA from inbred mouse strains. Lanes A through N are as for panel A. The probe corresponded to a 1.1-kbp *Hin*dIII fragment representing unique sequences at the 3′ end of unit II. The fragment was prepared from recombinant clone GR(40), provided by N. Hynes (13).

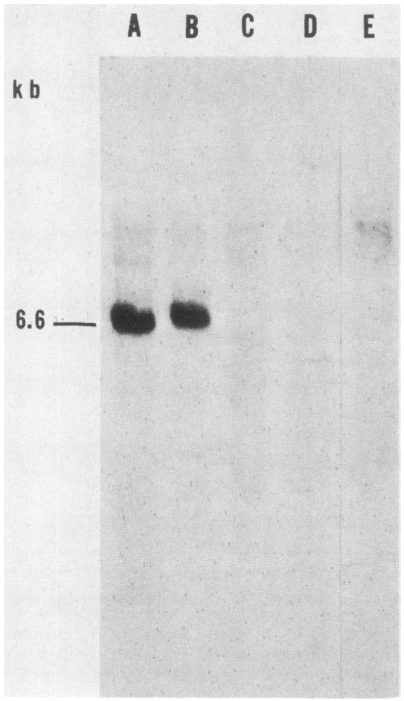

FIG. 2. Representative somatic cell hybrids digested with *Eco*RI and hybridized with the unit II 3′ flanking sequence probe. Lanes: A, NFS/N; B, HM6; C, HM7; D, HM31; E, E36 Chinese hamster.

*Eco*RI fragments. *Eco*RI-digested cellular DNAs from 13 common inbred strains of mice were examined by Southern blot analysis for the presence of MMTV-related fragments (Fig. 1A). All 13 inbred strains examined contained multiple MMTV proviral genomes. Except for the CE/J and NZB/BlNJ strains, each inbred strain appeared to contain the 8.3- and 6.6-kbp MMTV-related *Eco*RI fragments. However, the presence of these fragments did not establish that these strains contained the same proviral genome because of the frequent comigration of fragments related to different MMTV proviral genomes. To confirm the presence of unit II proviral genomes in these inbred strains, a probe was prepared which represented unique cellular sequences flanking the 3′ end of the endogenous GR mouse unit II genome. This probe detected the 6.6-kbp MMTV *pol-env*-LTR-related *Eco*RI fragment in all of the inbred mouse strains tested except CE/J and NZB/BlNJ (Fig. 1B). CE/J, NZB/BlNJ, and the feral *Mus musculus musculus* strain Czech II mouse DNAs contained a 4.9-kbp cellular *Eco*RI fragment which did not contain an MMTV proviral genome. The Czech II strain lacks endogenous MMTV genomes (3).

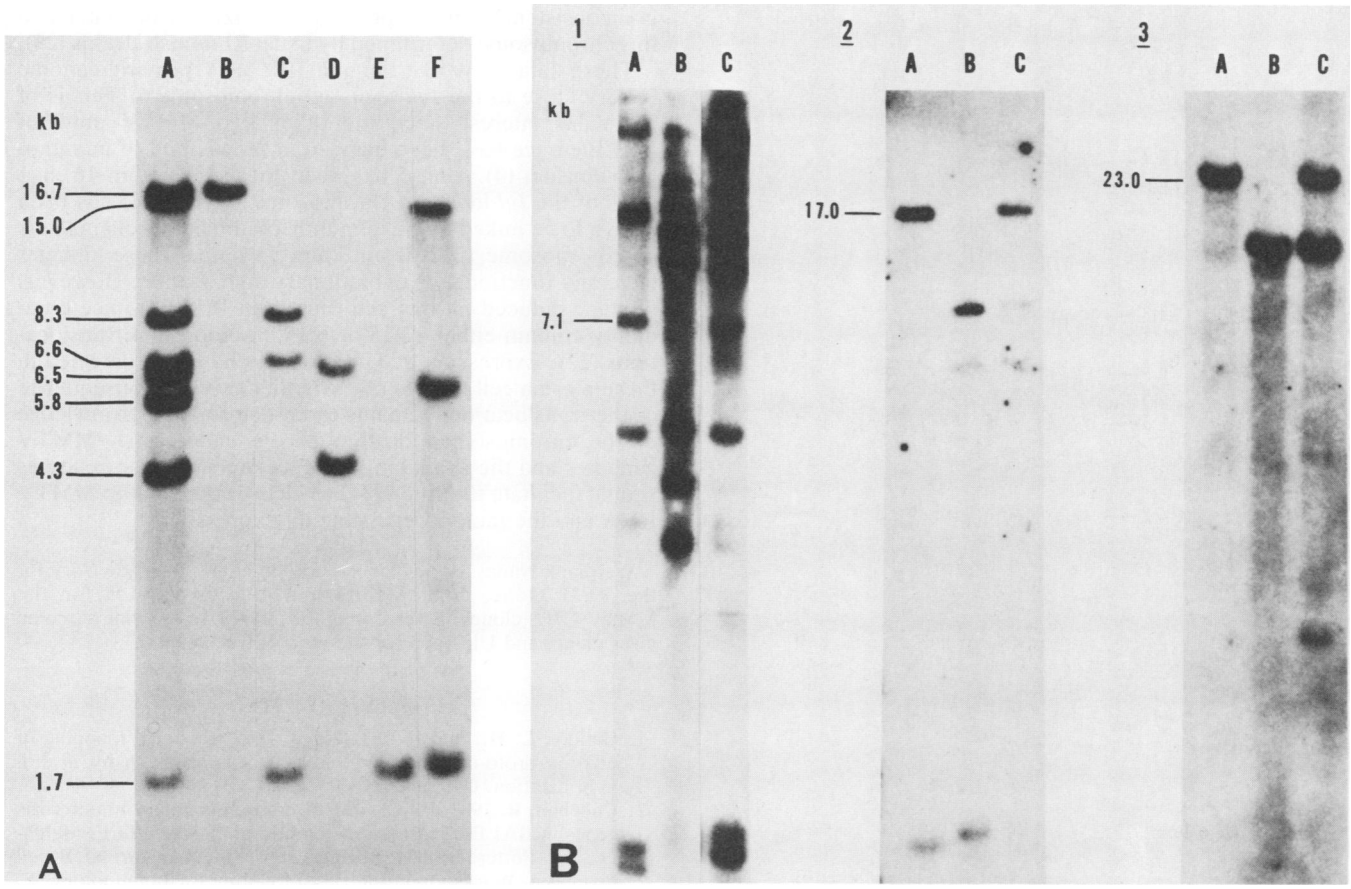

FIG. 3. (A) Segregating MMTV units from (C3H/OuJ × Czech II) × Czech II N1 mice. Lanes: A, C3H/OuJ; B, 16.7-kbp unit only; C, units II and IX; D, *Mtv-1* only; E, unit IX only; F, 15.0- and 5.8-kbp units and unit IX. *Eco*RI-digested DNA was hybridized with the MMTV LTR probe. (B) Restriction fragment length polymorphism. Lanes (all panels): A, C3H/OuJ; B, Czech II; C, C3H/OuJ × Czech II F_1 mice. Panel 1, *Eco*RI-digested DNA hybridized with an *Igk* probe corresponding to a 0.97-kbp *Pst*I fragment of recombinant clone Vk21, provided by S. Cory. Panel 2, *Bam*HI-digested DNA hybridized with a v-*raf* probe corresponding to a 1.0-kbp *Eco*RI-*Xma*III fragment of recombinant clone p171, provided by U. Rapp (25). Panel 3, *Pst*I-digested DNA hybridized with a v-K*ras* probe corresponding to a 1.0-kbp *Hin*cII fragment of recombinant clone pKMSV, provided by E. Chang (5).

The presence of this endogenous MMTV genome in several inbred mouse strains is consistent with a previous report that many European and American inbred mouse strains appear to have a common ancestor (8, 16). It has been shown that the unit II proviral genome is integrated into a member of the *Bam*HI family of repetitive sequences (7). Additional fragments detected in CE/J and Czech II mouse DNA with the unit II flanking region probe may represent amplification or transposition of these sequences to other cellular sites.

The chromosome containing the unit II MMTV genome was identified by screening *Eco*RI-restricted cellular DNA from 28 somatic cell hybrids. These hybrids were derived by fusing E36 Chinese hamster tissue culture cells with peritoneal or spleen cells from BALB/c, NFS/N, A/J, and C3H mice (16). Southern blot analysis with the unique flanking sequence probe showed a weak signal with Chinese hamster cellular DNA and a strongly hybridizing 6.6-kbp fragment in several hybrids (Fig. 2). Chromosome 6 showed the best correlation with these cellular sequences, indicating that the unit II provirus is present on this chromosome (Table 1).

TABLE 2. Segregation frequencies of units II and IX and chromosome 6 markers

Test locus	% Recombination ± SD (no. tested) with marker locus		
	Igk	*Raf*	K*ras-2*
Unit II	0[a] (85)	10 ± 4.3 (49)	33 ± 6.6 (51)
Unit IX	44 ± 5.1 (94)	39 ± 7.2 (46)	54 ± 7.2 (48)
Igk		10 ± 4.3 (49)	33 ± 6.6 (51)
Raf			29 ± 6.6 (51)

[a] $P < 0.035$, by the method of Bickel and Doksum as described by Louis (17). Other statistical analysis was done as previously described (10).

To confirm this result and further localize this MMTV proviral genome on mouse chromosome 6, liver DNA from (C3H/OuJ × Czech II) × Czech II backcross mice was examined. *Eco*RI-restricted C3H/OuJ cellular DNA contained eight MMTV-related fragments (Fig. 3A, lane A). Analysis of *Eco*RI-restricted cellular DNA from 95 backcross mice (Fig. 3A) revealed complete cosegregation of the 15.0- and 5.8-kbp fragments, the 8.3- and 6.6-kbp fragments (unit II), and the 6.5- and 4.3-kbp fragments (*Mtv-1*), which is consistent with previous reports (4, 20, 24, 29). Previous genetic studies showed that the MMTV proviral genomes defined by the 15.0- and 5.8-kbp and 6.5- and 4.3-kbp *Eco*RI fragments are located on chromosomes 14 and 7, respectively (24, 30). The 16.7- and 1.7-kbp (unit IX) *Eco*RI fragments segregated independently (recombination frequency [*r*], 49 ± 5.2). Both of these fragments represent

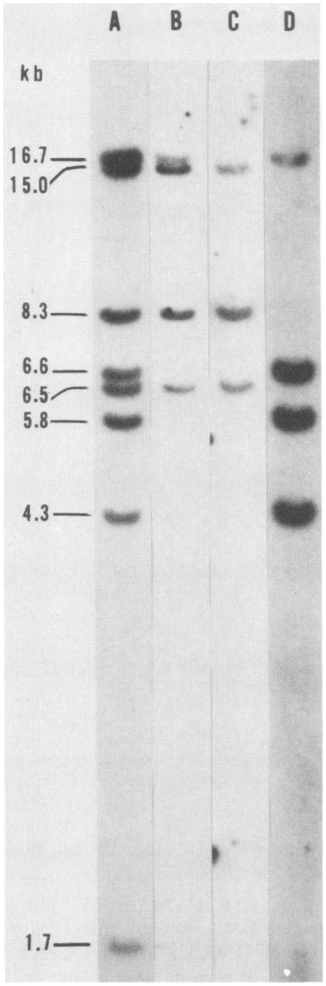

FIG. 4. MMTV-related sequences in *Eco*RI-digested cellular DNA from C3H/OuJ mice, hybridized with MMTV LTR probe (A), MMTV *gag* probe corresponding to a 2.0-kbp *Pst*I-*Xho*I fragment from GR(40) (B), MMTV *pol* probe corresponding to a 2.4-kbp *Xho*I-*Eco*RI fragment from GR(40) (C), and MMTV *env* probe corresponding to a 1.7-kbp *Pst*I fragment from MMTV(C3H) (D).

truncated MMTV proviral genomes. The 1.7-kbp fragment hybridized only with the MMTV LTR probe (Fig. 4, lane A) and has been reported to be located on chromosome 6 (29). The 16.7-kbp fragment hybridized with the MMTV LTR, *gag*, and *env* genes, but not with the *pol* gene (Fig. 4). This proviral genome was not observed in C3H/He cellular DNA (24); its origin and distribution in other C3H sublines are unknown.

The *Igk*, K*ras-2*, and *Raf* cellular genetic loci are known to be located on chromosome 6 (9, 12, 14, 26, 28). We monitored their segregation patterns relative to the unit II proviral genome by using restriction fragment length polymorphism to distinguish between parental alleles (Fig. 3B). Analysis of restricted cellular DNA from 85 backcross mice showed strong linkage between *Igk* and unit II (Table 2; $r = 0$ [$P < 0.035$]). Data on the linkage between unit II and *Raf* and between unit II and K*ras-2* (Table 2; $r = 10 \pm 4.3$ and $r = 33 \pm 6.6$, respectively), together with the localization of K*ras-2* to the distal end of chromosome 6 (1), provide the following gene order: (*Igk*–unit II)–*Raf*–K*ras-2*. Unit IX showed no linkage with the other chromosome 6 markers (Table 2). This is inconsistent with the previous localization of unit IX to this chromosome determined by using RI mouse strains (29).

These data show that the unit II MMTV proviral genome is very close to the *Igk* locus on chromosome 6. This is of particular interest since the other two MMTV units of BALB/c mice have been mapped in the vicinity of immunoglobulin loci (4). Unit I has been localized within 16 map units of the *Igl* locus on chromosome 16. Unit III has been shown to be linked to the immunoglobulin heavy chain locus on chromosome 12. It is not known whether these linkages have any functional or evolutionary significance. However, pristane-induced plasma cell tumors in BALB/c mice commonly contain either 12;15 or 6;15 chromosomal translocations (22). Expression of MMTV RNA has been detected in these plasma cell tumors (2). Whether this is a consequence of the translocations remains to be determined. Knowledge of the chromosomal location of the endogenous MMTV genomes and the availability of mice lacking these genomes should facilitate the study of the origin of the 1.6-kbp MMTV RNA and the factors regulating its expression.

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