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A New Common Integration Region (*int-3*) for Mouse Mammary Tumor Virus on Mouse Chromosome 17

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***Mus musculus* subsp. *musculus* (Czech II) mammary tumor DNA frequently contains an integrated proviral genome of the mouse mammary tumor virus (MMTV) within a specific 0.5-kilobase-pair region of the cellular genome (designated *int-3*). Viral integration at this site results in activation of expression of an adjacent cellular gene. We mapped *int-3* to mouse chromosome 17 by analysis of *Pst*I-restricted cellular DNAs from mouse-hamster somatic cell hybrids. Restriction analysis of cellular DNA from (C3H/OuJ × Czech II) × Czech II backcross mice established the gene order *T–H-2–int-3*. These results demonstrated that the *int-3* locus is distinct from two other common integration regions for mouse mammary tumor virus (designated *int-1* and *int-2*) in mammary tumor DNA and suggest that several cellular genes may be at risk for virally induced activation during mammary tumor development.**

Development of spontaneous mammary adenocarcinomas in the C3H and BR6 high-incidence inbred mouse strains is associated with chronic infection of the mammary gland by mouse mammary tumor virus (MMTV) (22). Development of mammary tumors in the C3H strain is pregnancy independent, whereas those in the BR6 strains are initially pregnancy dependent and subsequently progress, after several pregnancies, to hormone independence. MMTV, like other infectious retroviruses, is an insertional mutagen which appears capable of integrating at numerous sites within the cellular genome (21). Two cellular genetic loci (designated *int-1* and *int-2*) are frequently occupied by an MMTV proviral genome in mammary tumor DNA (13, 14). The *int-1* locus is primarily affected in C3H mice and feral *Mus cervicolor* subsp. *popaeus* pregnancy-independent mammary tumors, whereas MMTV integration into the *int-2* locus is detected early in pregnancy-dependent BR6 tumors (5, 13, 16). MMTV integration into these loci activates expression of previously silent cellular genes located within the respective loci (4, 13). The *int-1* and *int-2* loci are unrelated to each other or to the known proto-oncogenes (4, 12). However, the frequency with which they are activated by MMTV has led to the suggestion that their expression contributes to mammary tumorigenesis.

We have recently identified a new *int* locus (designated *int-3*) in MMTV-induced tumors of the feral *M. musculus* subsp. *musculus* strain (Czech II) (6). Czech II mice lack endogenous MMTV but carry an infectious MMTV [designated MMTV (Czech II)] which is transmitted by milk (1, 6a). These mice have a 12% incidence of pregnancy-independent type A mammary adenocarcinomas. In 30% of these tumors the *int-3* locus was occupied by an MMTV (Czech II) genome. Integration of a viral genome at this locus activates expression of a 2.4-kilobase (kb) species of RNA corresponding to adjacent cellular DNA sequences. Cellular DNA sequences related to the *int-3* transcribed region have been conserved in mammalian and avian species. This strongly suggests that the etiology of pregnancy-independent mammary tumors involves quantitative (and perhaps qualitative) activation of multiple cellular genes. To further define the relationship of this locus to the *int-1* and *int-2* loci, as well as the proto-oncogenes, we determined its location within the cellular genome.

The chromosomal location of the *int-3* locus was identified by screening *Pst*I-restricted cellular DNAs from 18 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/HeJ mice (8). The restricted cellular DNAs were blot hybridized with a unique 2.9-kb fragment of cellular DNA from the *int-3* locus (Fig. 1, probe A). Probe A reacted weakly with a 2.0-kb *Pst*I fragment of Chinese hamster DNA under stringent blot hybridization conditions but produced a strong signal with a 3.6-kb *Pst*I mouse DNA fragment (Fig. 2). Each of the hybrid cellular DNAs which contained mouse chromosome 17 also contained the 3.6-kb *Pst*I fragment (Table 1). One exceptional hybrid DNA (Fig. 2, lane G) which contained the 3.6-kb *Pst*I fragment did not appear by isozyme or karyotype analysis to contain mouse chromosome 17. Since probe A reacted weakly with this hybrid cellular DNA, it may be that only a minor fraction of cells contained mouse chromosome 17 or a translocated portion of this chromosome.

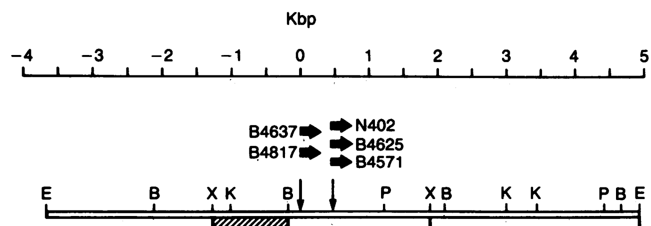

FIG. 1. Partial restriction map of the *int-3* locus. The restriction sites are indicated as follows: E, *Eco*RI; B, *Bam*HI; X, *Xba*I; K, *Kpn*I. The hatched box corresponds to a portion of the cellular transcribed region. The vertical arrows indicate sites of integration of MMTV (Czech II) proviral DNA. The horizontal arrows indicate transcriptional orientation. The open box corresponds to the *Xba*I-*Eco*RI restriction fragment designated probe A. Kbp, Kilobase pairs.

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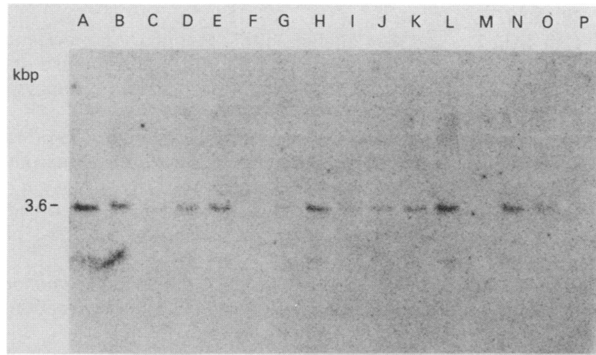

FIG. 2. *int-3*-related sequences in *Pst*I-digested cellular DNAs from (lanes) Czech II liver (A); hamster-mouse hybrids HM-36 (B), HM-58 (C), HM-15 (D), HM-57 (E), HM-23 (F), HM-35 (G), HM-27 (H), HM-34 (I), HM-37 (J), HM-22 (K), HM-44 (L), HM-17 (M), HM-6 (N), and HM-49 (O); and E36 Chinese hamster cells (P). High-molecular-weight DNA was prepared as previously described (1). *Pst*I-digested samples of DNA (10 μg) were electrophoretically separated on 0.8% agarose gels, transferred to nitrocellulose membranes, and hybridized under stringent conditions (1) with 2×10^6 to 5×10^6 cpm of ^{32}P-labeled probe A (prepared by nick translation) per ml. Kbp, Kilobase pairs.

Restriction fragment length polymorphisms (RFLPs) have frequently been observed in cellular DNAs from inbred strains of *M. musculus* subsp. *domesticus* and *M. musculus* subsp. *musculus* (Czech II) (1, 17). To confirm the apparent presence of *int-3* on chromosome 17 and to define its position relative to other genetic loci, we examined the first-backcross generation of [(C3H/OuJ × Czech II) × Czech II]N_1 mice for segregation of C3H-specific RFLPs. Seventy-five N1 mice were typed by restriction enzyme analysis for segregation of the *T*, *H-2* (MHC), and *int-3* loci. Figure 3 shows C3H- and Czech-II-specific RFLPs in restricted cellular DNAs from parental and F_1 mice. The Czech-II-derived *int-3* probe A reacted with a 4.0-kb fragment in C3H/OuJ and a 2.3-kb fragment of *Pvu*II-digested Czech II cellular DNA (panel A). The *I-A*-specific probe from the *H-2* locus detects a 9.0-kb *Bgl*II fragment in Czech II DNA and a 5.0-kb fragment in C3H DNA (3). The *H-2* locus is located 12 to 15 centimorgans distal to the *T* locus (20). The *T* locus in this study is defined by the Tu66 recombinant DNA probe and corresponds to cellular DNA sequences which are located near the centromere on chromosome 17 (18). The Tu66 probe reacted with 1.8- and 1.5-kb *Taq*I restriction fragments in C3H cellular DNA (panel C). The backcross mouse DNA contained either the Czech II RFLP or a heterozygous pattern consisting of both RFLPs. Chi-square analysis of the genotypes in N_1 mice demonstrated that the *T*, *H-2*, and *int-3* loci did not segregate independently ($P < 0.001$). The recombination frequencies among the three genetic loci are shown in Table 2. Since there is a stronger linkage between the *int-3* and *H-2* loci (recombination = 11 ± 4 centimorgans) than between the *int-3* and *T* loci (recombination = 19 ± 5 centimorgans), the apparent gene order is *T*–*H-2*–*int*-3. These data confirm the presence of *int-3* on chromosome 17 and further distinguish this locus from (i) the

TABLE 1. Correlation between *int-3* and mouse chromosomes in 18 somatic cell hybrids

Mouse chromosome	No. of hybrid clones[a] showing *int*/chromosome retention: +/+	−/−	+/−	−/+	% Discordant
1	7	2	6	2	47
2	8	2	6	2	44
3	5	3	6	1	47
4	3	2	10	2	71
5	2	4	12	0	67
6	6	2	7	2	53
7	11	1	3	3	33
8	3	4	10	0	59
9	4	4	9	0	53
10	1	4	13	0	72
11	0	4	13	0	76
12	6	2	5	2	47
13	4	2	7	2	60
14	5	4	4	0	50
15	9	0	2	4	40
16	6	3	7	1	47
17	13	3	1	0	6
18	8	3	4	1	31
19	5	2	8	2	59
X	7	4	5	0	31

[a] Fifteen hybrids were typed for mouse chromosomes by direct karyology by using Giemsa-trypsin banding; three hybrids were typed for specific marker loci (8).

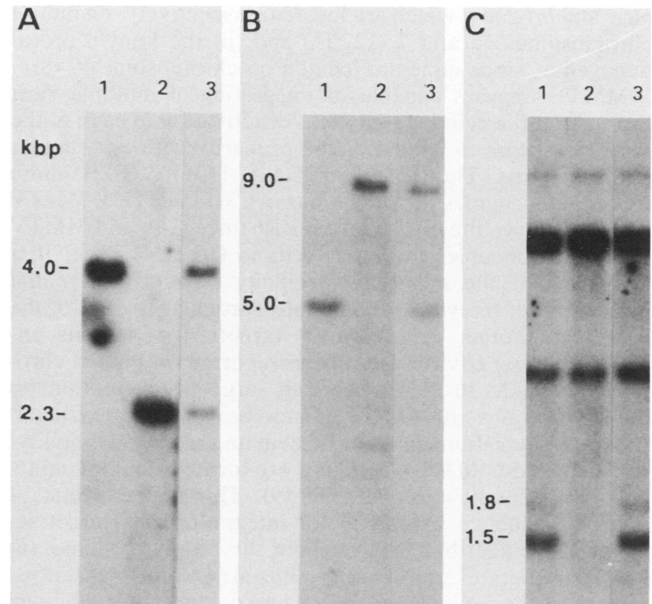

FIG. 3. Restriction fragment length polymorphisms in DNAs of C3H/OuJ (lane 1), Czech II (lane 2), and (C3H/OuJ × Czech II)F_1 (lane 3) mice. *Pvu*II-digested DNA was hybridized with *int-3* probe A (panel A). *Bgl*II-digested DNA was hybridized with the *I-A*-specific *H-2* probe (panel B) provided by D. Singer. *Taq*I-digested DNA was hybridized with Tu66 probe provided by L. M. Silver (panel C). Molecular sizes in kilobase pairs (kbp) are indicated to the left of each panel.

TABLE 2. Segregation frequencies of *int-3* and chromosome 17 markers[a]

Locus	Mean (± SEM) recombination (centimorgans) (no. of animals scored) with: *H-2*	*T*
int-3	11 ± 4.0 (66)	19 ± 5.0 (54)
H-2		15 ± 5.0 (48)

[a] Statistical analysis was done as described by Green (6).

int-1 and *int-2* loci which are located, respectively, on mouse chromosomes 15 and 7 (12, 15) and (ii) the known proto-oncogenes, since none are located on chromosome 17 (8).

MMTV appears capable of integrating at multiple sites throughout the cellular genome, yet activation of each of the *int* loci appears to be associated primarily with a particular strain of virus. Thus, in tumor DNA, MMTV (C3H) more frequently occupies the *int-1* locus in C3H mice (13), MMTV (RIII) occupies the *int-2* locus in BR6 mice (14), and MMTV (Czech II) occupies the *int-3* locus in Czech II mice. It is possible that the apparent specificity reflects preferential integration of the viral genome into particular regions of the cellular genome. High-affinity targets for baboon endogenous type C virus have been reported on human chromosome 6 (2, 10, 11). Although targeted integration by different strains of MMTV cannot be formally excluded, several observations make this seem an unlikely possibility. (i) Endogenous MMTV genomes are located on a minimum of eight different chromosomes (9). This suggests that, if there is sequence specificity for integration, the target sequences are distributed throughout the cellular genome. (ii) MMTV integrates at numerous sites around the transcribed regions of the *int* loci, yet there is no evidence of homology between different restriction fragments representing the unrearranged *int* loci. (iii) The *int-1* locus on *M. cervicolor* subsp. *popaeus* mammary tumor DNA is frequently occupied by an MMTV-related viral genome (*M. cervicolor* mammary tumor virus) which has diverged significantly from the MMTV genomes of *M. musculus* (19). We favor an alternative possibility in which activation of expression of the *int* loci by MMTV integration provides tumor cells with a selective advantage (13, 14). The *int-3* locus could then represent a new member in a family of mammary tumor-associated cellular genes. The basis of the specificity with which different MMTV strains activate particular *int* loci is unknown but raises the possibility that additional *int* loci will be discovered in mammary tumors induced by other feral MMTV strains (6a).

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