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Insertional mutagenesis identifies a member of the *Wnt* gene family as a candidate oncogene in the mammary epithelium of *int-2/Fgf-3* transgenic mice

(mouse mammary tumor virus/tumor progression/breast cancer)

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ABSTRACT **Transgenic mice harboring the *int-2/Fgf-3* protooncogene under transcriptional control of the mouse mammary tumor virus (MMTV) promoter/enhancer exhibit a dramatic, benign hyperplasia of the mammary gland. In one *int-2* transgenic line (TG.NX), this growth disturbance is evoked by pregnancy and regresses after parturition. Regression of hyperplastic mammary epithelium is less complete after successive pregnancies, and, within 10 months, most TG.NX mice stochastically develop mammary carcinomas that are transplantable in virgin, syngeneic mice. To identify genes that cooperate with *int-2* in cell transformation, we infected TG.NX transgenic mice with MMTV. In a cohort of 14 animals, most mammary tumors represented clonal or oligoclonal outgrowths harboring one to five proviral MMTV integrants. Eight of 35 (23%) MMTV$^+$ tumors exhibited proviral insertion at the *Wnt-1* locus. No provirus was detected at the *int-2*, *int-3*, or *Wnt-3* loci. By Southern analysis, two tumors had proviral insertions at the same genomic location, which was mapped to chromosome 15. Cloning of this *int* locus identified an additional member of the *Wnt* gene family. The predicted 389-amino acid protein is most closely related to zebrafish *Wnt-10a* (58% amino acid identity over 362 residues) and, based on homology analysis, was designated *Wnt-10b*. This newly discovered *Wnt* family member was expressed in the embryo and mammary gland of virgin but not pregnant mice and represents a candidate collaborating oncogene of *int-2/Fgf-3* in the mammary epithelium.**

int-2, a fibroblast growth factor family member (*Fgf-3*), was originally identified as a common integration site of mouse mammary tumor virus (MMTV) in virally induced mammary tumors (1). Similar to other MMTV *int* loci, proviral integration inappropriately activates expression of *int-2* in the mammary gland. This activation has been associated with pregnancy-dependent mammary tumors in, for example, the BR6 and GR strains of mice (2, 3). The *int-2* gene is normally expressed in the developing embryo but not in the adult mouse (4). While its cellular function remains to be defined, *in vivo* expression analysis as well as derivation of homozygous *int-2* null mouse mutants have suggested roles in cell migration, tissue induction, or neuronal cell differentiation or survival (5, 6).

In an effort to characterize the consequences of inappropriate expression of *int-2* in the mammary gland *in vivo*, we and others have derived transgenic mice carrying the wild-type *int-2* gene under the transcriptional control of the MMTV long terminal repeat (LTR) (7–9). In our initial report, we described two such transgenic lines (7). One, TG.NX, bears an *int-2* cDNA fused to a truncated MMTV LTR. Pregnancy induces a marked, well-differentiated benign hyperplasia of the mammary gland, which is functional in that TG.NR mothers are able to nurse their young. Notably, postlactational glands fail to regress even months after parturition. Although females may develop cystic lesions after long latencies, these are benign.

By contrast, a second transgenic line, TG.NX, contains a full-length MMTV LTR fused to the murine *int-2* cDNA. Similar to analogous lines described elsewhere (8, 9), the mammary glands of pregnant TG.NX females exhibit markedly abnormal ductal hyperplasia and extensive stromal proliferation. Consistent with these findings, TG.NX mothers are unable to nurse their young. After parturition, the mammary glands regress; however, regression is less complete after successive pregnancies, and most animals develop stochastic mammary carcinomas within 10 months. These tumors are transplantable in virgin, syngeneic mice (7, 10).

The focal occurrence of tumors in TG.NX mice implies that *int-2* is not sufficient for full transformation of mammary epithelial cells. To identify additional genes that cooperate with *int-2*, we infected TG.NX mice with MMTV and analyzed resultant mammary tumors for common proviral integration sites. Recently, a similar analysis using transgenic mice harboring the *Wnt-1* (formerly *int-1*) gene showed frequent viral activation of *int-2* (11). *Wnt-1*, the first cloned MMTV insertion site, represents a class of developmental genes with homology to the *Drosophila* segment polarity gene wingless (12, 13). Frequent coactivation of *Wnt-1* and *int-2* in virally induced tumors as well as cooperative tumorigenic effects of these protooncogenes in bitransgenic mice harboring both genes have implied a collaborative action between these two classes of growth factors in mammary neoplasia (14, 15).

This study reports the findings with 14 TG.NX animals infected with MMTV. A newly discovered *int* locus, which appears to be a member of the *Wnt* family, is described, together with a preliminary characterization of this gene.¶

MATERIALS AND METHODS

Transgenic Mice. The TG.NX mouse line contains the MMTV LTR fused to the wild-type murine *int-2* cDNA (7) and was derived from FVB/N inbred mice (Taconic Farms).

MMTV Infection. Virgin female mice (7–12 weeks old) were injected i.p. with 10^7 live EH-2 cells and bred continuously to enhance viral infection. EH-2 is a rat XC cell line that produces a pathogenic hybrid MMTV consisting primarily of MMTV(C3H) sequences (16).

Analysis for Proviral Integrants. High molecular weight DNA was isolated from tumor tissue by standard procedures

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Abbreviations: MMTV, mouse mammary tumor virus; LTR, long terminal repeat.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. U20658).

(17). To assay for MMTV provirus, *Bgl* II-digested DNAs were hybridized with a 1.2-kb *Bam*HI *env* probe (16), which detects a 4.2-kb internal *Bgl* II fragment diagnostic for MMTV(C3H). To analyze the flanking genomic region, *Hin*dIII-digested DNAs were hybridized with the identical *env* probe.

MMTV(C3H)$^+$ tumors were analyzed for viral integration at known *int* loci. *Wnt-1*: *Eco*RI-digested DNA was hybridized with a 1870-bp *Hin*dIII/*Xba* I fragment from pBSKS+ *int-1*, derived from clone 26 (gift of J. Mason, University of California, San Francisco; ref. 18) and a genomic fragment extending 2.3 kb 5′ from the *Bam*HI site in exon 2 of *Wnt-1*. *int-2*: *Hin*dIII-digested DNA was probed with the 2.4-kb *Hin*dIII fragment from pKC3.9 (19). *int-3*: *Eco*RI-digested DNA was hybridized with a 750-bp *Pst* I fragment from pC241L (gift of D. Gallahan and R. Callahan, Oncogenetics Section, National Institutes of Health; ref. 20). *Wnt-3*: *Kpn* I-digested DNA was hybridized with a 1.3-kb *Bam*HI/*Hin*dIII fragment from pBG14 (gift of P. Salinas, Stanford University; ref. 21).

Inverse PCR. Genomic DNA (200 ng) digested with the appropriate restriction enzyme was self-ligated in a standard 200-μl ligation reaction mixture at 14°C overnight. Ligated DNA was amplified in a 100-μl PCR mixture containing 1× PCR buffer (Perkin–Elmer; 1.5 mM $MgCl_2$), 250 μM dNTPs, 2.5 units of *Taq* polymerase, and 50 pmol of each oligonucleotide. For the first round of PCR, primers i1 from the 5′ end of the MMTV LTR (5′-CCTTGGTGGGAAACAACCCCT-TGGCTGCTT-3′) and i3a from the 3′ end of the LTR (5′-CGAGGATGTGAGACAAGTGGTTTCCTGACT-3′) were used. Cycle conditions were 95°C for 1 min, 58°C for 2 min, 72°C for 3 min 40 times. Two microliters of this reaction mixture was then subjected to heminested PCR with primers i1 and i3b (5′-GGTTCTGATCTGAGCTCTGAGTGTTC-TATT-3′), which lies 16 bp 3′ of i3a in the LTR. Cycle conditions were as described above.

RESULTS

MMTV Infection of TG.NX Mice Gives Rise to Clonal and Oligoclonal Tumors. A cohort of 14 TG.NX virgin female mice 7–12 weeks old was infected with MMTV(C3H) and bred to enhance viral replication. A majority of mammary tumors arising in these animals were positive for the diagnostic 4.2-kb *Bgl* II MMTV(C3H)-specific band using an *env* probe (Fig. 1*A*). Tumors occurred from 108 to 335 days postinfection, similar to uninfected TG.NX animals (Table 1). Notably, most MMTV(C3H)$^+$ tumors arose <175 days after infection, whereas the majority of uninfected tumors occurred >240 days postinfection. In addition, MMTV(C3H)$^+$ tumors occurred in animals harboring significantly more tumors per animal. The histopathology of tumors containing proviral integrants conformed to types previously ascribed to MMTV (Dunn types A and B; ref. 22) while most MMTV$^-$ tumors were papillary or adenosquamous carcinomas typical of uninfected TG.NX mice.

Genomic Southern analysis demonstrated that MMTV(C3H)$^+$ mammary tumors contained one to five unique flanking bands (Fig. 1*B*; data not shown). Thus, these tumors represented clonal or oligoclonal outgrowths. Eight of thirty-five (23%) tumors exhibited proviral integrants at *Wnt-1* (Table 1). No alterations in the *int-2*, *int-3*, and *Wnt-3* genomic regions were observed with probes specific for these loci (data not shown).

Identification of a Common MMTV Integration Site. Two independent tumors from different animals (NX 6370 and NX 7182) exhibited cellular flanking bands of the same size on a Southern blot of *Hin*dIII-digested tumor DNA hybridized with the *env* probe. Subsequent analysis with other restriction enzymes revealed an identical pattern in both samples (Fig. 2). Thus, within the resolution of Southern analysis, two proviruses in independent events integrated at the same location in genomic DNA.

Cloning of the *int* Locus. The integration site in tumor NX 6370 was cloned by inverse PCR. Briefly, it was found that digestion of tumor DNA with *Bgl* II and hybridization with a MMTV LTR probe yielded a 3.0-kb band, slightly larger than a 2.7-kb endogenous band. Thus, *Bgl* II-digested tumor DNA was electrophoresed and the 3-kb region was excised. DNA was eluted from the gel fragment, self-ligated, and subjected to inverse PCR using MMTV LTR oligonucleotide primers oriented outward from each end of the enhancer. Heminested PCR amplification of this initial product yielded the fragment of expected size, which was subcloned and sequenced. While a homology search in the GenBank data base (National Center for Biotechnology Information) did not identify any known gene, a direct match was found to a MMTV integration site in a tumor designated W26, in which *int-2* was also activated by MMTV (23). The W26 provirus was located 766 bp distal to the NX 6370 integration site in the opposite orientation.

Primers derived from the NX 6370 flanking sequence were then used to amplify the MMTV integration site in tumor NX 7182. Sequencing of this PCR product showed that the pro-

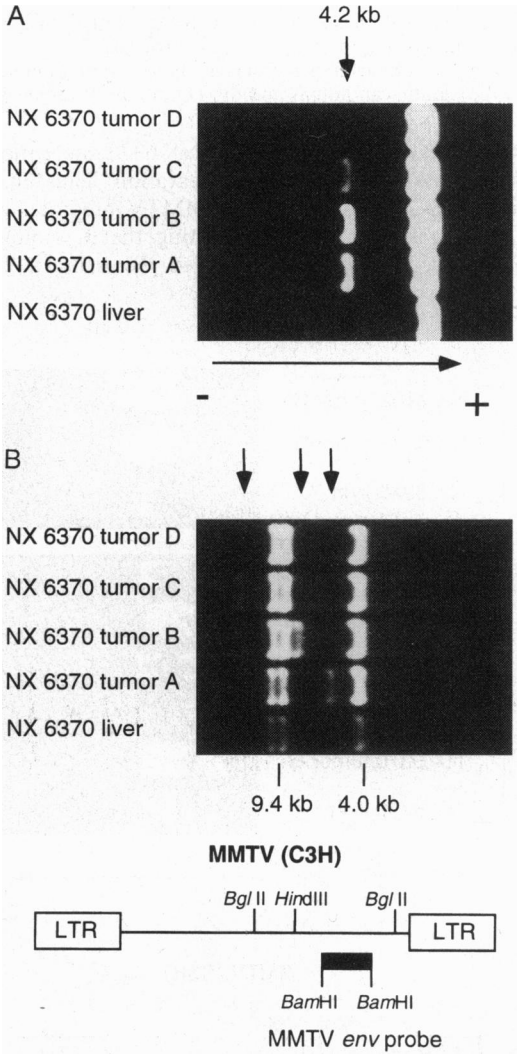


FIG. 1. Evidence for integrated MMTV(C3H) provirus in mammary tumors of infected TG.NX mice. (*A*) Southern blot of *Bgl* II-digested genomic DNA from liver (germ-line configuration) and four tumors from animal NX 6370 hybridized with the MMTV *env* probe depicted. The 4.2-kb band is diagnostic for MMTV(C3H). The smaller molecular weight band represents endogenous viral sequences. (*B*) Tumor DNAs digested with *Hin*dIII and hybridized with the *env* probe. Arrows indicate unique bands demonstrating clonal proviral integration events.

Table 1. Summary of results from MMTV(C3H) infection of TG.NX mice

Animal	Age at death, days*	Time from infection to death, days*	No. of tumors	No. of MMTV$^+$ tumors†	No. of *Wnt-1*$^+$ tumors	Histopathology‡
1. NX 6363	210	132	1	0/1	ND	Papillary tumor
2. NX 6371	207	132	5	2/4	ND	Secretory alveolar Dunn type A (1)
3. NX 6370	210	132	4	3/4	2/4	Secretory alveolar Dunn type A (1)
4. NX 9760	163	108	1	ND	ND	Mixed Dunn types A and B
5. NX 2198	193	108	7	7/7	1/7	Dunn type A (1)
6. NX 7181	243	173	8	8/8	3/8	Dunn type B (5)
7. NX 7182	228	173	5	5/5	2/5	Dunn type B (4)
8. NX 7183	263	173	3	2/3	0/3	Dunn type B (2)§
9. NX 92892	313	243	2 (3)¶	0/2	ND	Adenosquamous carcinoma and adenocarcinoma
10. NX 92894	313	243	1	0/1	ND	Papillary adenocarinoma
11. NX 92895	313	243	3	0/2	ND	Hyperplasias
12. NX 9779	298	243	1 (1)¶	0/1	ND	Papillary adenocarcinoma
13. NX 92896	313	243	2 (4)¶	1/1	0/1	Adenocarcinoma
14. NX 2181	399	335	10	7/10	0/7	Keratoacanthoma (6); Dunn type B (1)‖

*Animals were sacrificed at the latest times possible, before distress to the host and when it was judged unlikely that additional tumors would arise before death.
†Number of tumors with integrated MMTV(C3H) provirus. Not all tumors gave sufficient material for genomic DNA analysis; ND, not determined.
‡Number of tumors subjected to histopathological analysis is indicated in parentheses; MMTV(C3H)$^+$ unless otherwise noted.
§One tumor did not harbor MMTV(C3H) provirus.
¶Numbers in parentheses indicate masses that were predominantly keratin debris.
‖ Four of the keratoacanthomas and the Dunn type B tumor were MMTV(C3H)$^+$.

virus was located 150 bp distal to the NX 6370 integration site (916 bp from W26) in the same orientation. Thus, in three independent *int-2* mammary tumors, MMTV integrated within <0.92 kb of genomic DNA, suggesting that a biologically relevant *int* locus had been identified. By using a 270-bp *Bam*HI/*Xho* I flanking genomic probe (designated probe B), structural analysis of this *int* region was extended by screening a mouse genomic library (129 strain; Stratagene) and obtaining two genomic clones, which were partially sequenced and restriction mapped (see Fig. 4*A*).

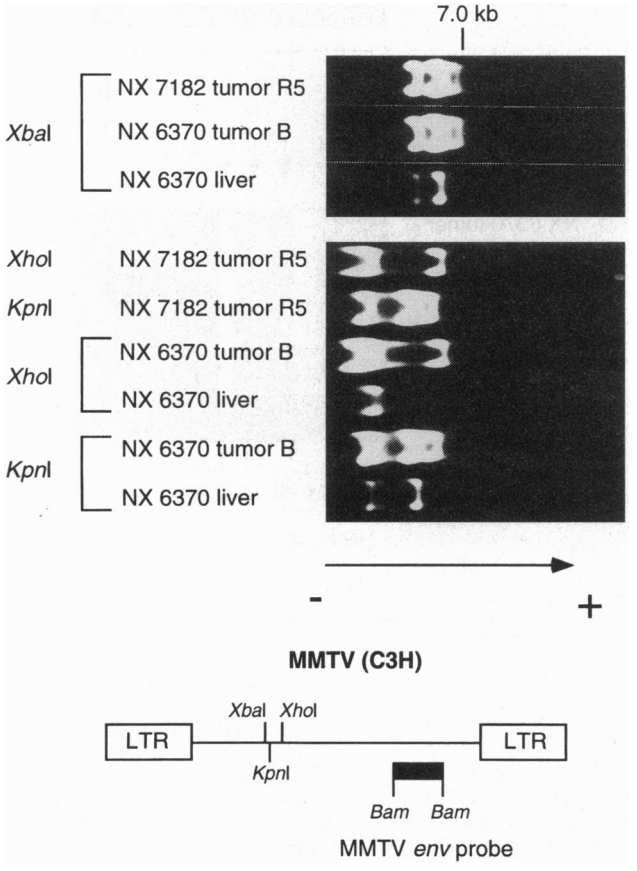


FIG. 2. Two independent mammary tumors harbor MMTV(C3H) at the same location in genomic DNA. Southern blot of liver (germline configuration) and tumor DNAs digested with restriction enzymes as shown and hybridized with the *env* probe.

The *int* Site Maps to Chromosome 15. Oligonucleotide primers flanking a 22-bp CA repeat in genomic sequence flanking the insertion site detected a small, ≈10-bp repeat length polymorphism in C57BL/6 vs. DBA strains of mice. These primers were used to amplify genomic DNA from the BXD recombinant inbred strain panel (The Jackson Laboratory). The strain distribution pattern of the *int* locus in the BXD cross is given in Table 2 and showed 0/26 recombinants with *Pmv-42*, a nonecotropic murine leukemia provirus located at the distal end of chromosome 15 (24). The strain distribution pattern of *Pmv-42* in a different cross (AKXD; The Jackson Laboratory) shows 0/24 recombinants with that of *Wnt-1* (B. Taylor, personal communication), indicating tight linkage. We did not detect *Wnt-1* transcripts in the NX 6370 and NX 7182 tumors (data not shown).

The *int* Site Represents a Transcribed Locus. To assess whether cellular sequences from the *int* locus were present in mRNA, a Northern blot of poly(A)$^+$ RNA from various tissues of MMTV$^-$ (uninfected) mice was hybridized with probe B (Fig. 3). An ≈3.6-kb transcript was detected in the mammary gland of virgin but not pregnant mice as well as in near-term embryos. Highest expression was seen in an *int-2* mammary

Table 2. Strain distribution pattern of the *int* locus in the C57BL/6 × DBA (BXD) recombinant inbred strain panel

Chromosome 15 marker	Strain distribution pattern
Rsp-7	BD DD BB BDBDBB DBBDBBD DDBDBD
Aft-4	BD DB DD BBDDDB DDDDBD DBDDB
Pdgfb	BD DB DD BBDD B DDDDDBDB DDBDDB
Cyp2d9	BD DB DD BDD B DDDDDBDB DDBD
Pmv-42	BB BB DD BDDBBB DBDDBBDD DBDDBD
int	BB BB DD BDDBBB DBDDBBDD DBDDBD
Hox-3	DB DB DD BDDBBB DBDDBBDB DBDDBD
Spt-2	DB DB DD B DBBB DDDDBBDB DBDDBD

Chromosome 15 markers are given from proximal (top) to distal (bottom). B, C57BL/6 allele; D, DBA allele.

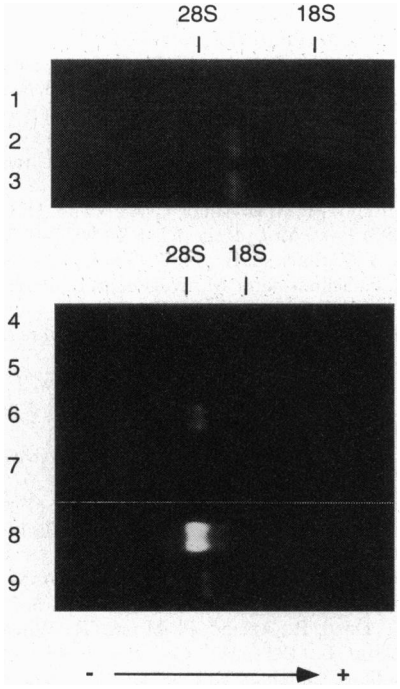


FIG. 3. A 270-bp *Bam*HI/*Xho* I genomic probe detects a low-abundance 3.6-kb transcript in normal and tumor tissues. Northern blot analysis with polyadenylylated mRNA; film was exposed for 11 days (lanes 1–3) and 9 days (lanes 4–9). Lanes: 1, mammary gland from 17-day pregnant (+/+) mouse; 2, virgin mammary gland (+/+); 3, day 14.5 embryo (+/+); 4, adult brain (+/+); 5, adult testis (+/+); 6, near-term embryo from *int-2*/+ mother; 7, pregnant mammary gland (*int-2*/+); 8, mammary tumor, uninfected TG.NX mouse; 9, virgin mammary gland (*int-2*/+).

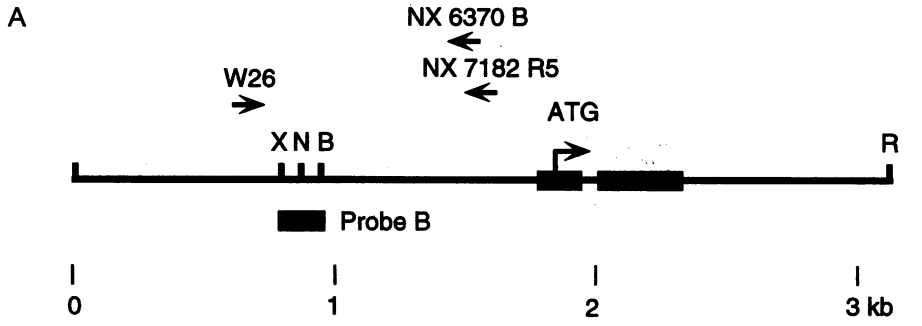
tumor. Notably, Fig. 3 represents an extended exposure of poly(A)$^+$ RNA Northern blots, underscoring the low abundance of this message. Using limited amounts of available total RNA from tumors NX 6370 and NX 7182 (10 μg), we were not able to detect the 3.6-kb transcript by Northern analysis.

The Transcript from the *int* Locus Encodes a Member of the *Wnt* Gene Family. Probe B was used to screen a 17-day murine embryo cDNA library (Clontech). Two independent clones were obtained and sequenced bidirectionally, yielding a 1.16-kb open reading frame (Fig. 4). Comparison with the Geninfo BLAST Network Service protein data base identified this as a newly discovered member of the *Wnt* family of protooncogenes, which corresponds to a partial 384-bp sequence identified by reverse transcriptase PCR (25). Highest-scoring amino acid sequence pairs were formed with *Wnt-10a* from zebrafish (58% identity over 362 residues) and *Wnt-10* from *Xenopus* (67% identity over 128 residues) (26, 27). Notably, the N terminus of this *Wnt* gene is divergent from that of zebrafish *Wnt-10a*. Based on homology comparisons, we designate this family member *Wnt-10b* (ref. 28; A. Sidow, personal communication). A virtually identical gene sequence was obtained by one of us (G.M.S.) in independent experiments by degenerate PCR and will be reported elsewhere.

DISCUSSION

int-2 is unique among murine mammary oncogenes in that its misexpression is associated with a pregnancy-dependent growth disturbance. Whereas *Wnt-1* is the dominant target for MMTV activation in pregnancy-independent mammary tumors (e.g., in C3H mice), *int-2* activation is more frequent in mouse strains exhibiting pregnancy-dependent lesions (29, 30). These observations have been strikingly confirmed in *int-2* transgenic mice and suggest that *int-2* is by itself insufficient to fully transform mammary epithelial cells. To identify an additional collaborating gene(s), we chose to exploit the selective tumorigenic potential of MMTV. Infection of TG.NX females resulted in (*i*) earlier onset of mammary tumors; (*ii*) increased number of tumors per animal; (*iii*) altered tumor histology; and (*iv*) frequent proviral integration at *Wnt-1*, a known collaborator of *int-2*.

In two independent tumors, MMTV integration occurred within a 150-bp segment of genomic DNA. Cloning of the adjacent cellular region identified a member of the *Wnt* family as the putative target. Notably, our genomic sequence corresponded to a MMTV integration site in the GenBank data base that had been previously described in a mammary tumor in which *int-2* was also activated (23). These data underscore the known cooperativity between *int-2* and *Wnt-1* and parallel reciprocal data in which MMTV-infected *Wnt-1* transgenic mice exhibit activation of *int-2* as well as the related *hst/Fgf-4* gene in mammary tumors (11). The molecular basis of interaction between these two classes of growth factors remains to be defined. Recently, intriguing experiments in *Xenopus* mesoderm induction have shown that *XWnt-8* can act as a "competence



B

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MLEEPRSRPPPLGLAGLLFLALFSRALSNEILGLKLPGEPPLTANTVCLTLSGLSKRQLG   60
LCLRSPDVTASALQGLHIAVHECQHQLRDQRWNCSALEGGGRLPHHSAILKRGFRESAFS  120
FSMLAAGVMHAVATACSLGKLVSCGCGWKGSGEQDRLRAKLLQLQALSRGKTFPISQPSP  180
VPGSVPSPGPQDTWEWGGCNHDMDFGEKFSRDFLDSREAPRDIQARMRIHNNRVGRQVVT  240
ENLKRKCKCHGTSGSCQFKTCWRAAPEFRAIGAALRERLSRAIFIDTHNRNSGAFQPRLR  300
PRRLSGELVYFEKSPDFCERDPTLGSPGTRGRACNKTSRLLDGCGSLCCGRGHNVLRQTR  360
VERCHCRFHWCCYVLCDECKVTEWVNVCK                                  389
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FIG. 4. Three independent mammary tumors arising in virally infected animals harboring an activated *int-2* gene exhibit MMTV provirus integrated within <0.92 kb of genomic DNA. (*A*) Schematic diagram of the 5′ end of the *int* locus is shown. Arrows indicate site and orientation of integrated proviruses in the mammary tumors indicated. Solid boxes denote exons, with the arrow depicting the putative ATG translation initiation codon of the sequence in *B*. The 270-bp *Bam*HI/*Xho* I probe described in the text is shown (probe B). X, *Xho* I; N, *Not* I; B, *Bam*HI; R, *Eco*RI. (*B*) Complete amino acid sequence derived from a 1167-bp open reading frame in two independent cDNAs.

modifier" that alters the response of blastula animal caps to the *int-2*-related basic fibroblast growth factor (31).

The *Wnt* gene described here encodes a 389-amino acid protein most closely related to zebrafish *Wnt-10a*; however, it probably does not represent murine *Wnt-10a* since the N termini are divergent. Our cDNA corresponds to a 384-bp partial sequence derived from reverse transcriptase PCR of mouse embryonic RNA (25), which was designated *Wnt-12* in that reference. Based on full sequence analysis, however, which demonstrated closest relatedness to an unpublished salamander *Wnt-10b* sequence (A. Sidow, personal communication), and in accordance with the standard *Wnt* nomenclature (28), we propose that this gene be designated *Wnt-10b*. Exceedingly low *Wnt-10b* expression was detected in the developing embryo and in the mammary gland of virgin but not pregnant mice. Notably, highest expression was found in a mammary tumor from an uninfected ($MMTV^-$) TG.NX animal. The low abundance of this message prohibited detection of transcripts in the limited amount of total RNA available from tumors NX 6370 and NX 7182.

These studies demonstrate the potential for viral mutagenesis to detect loci implicated in cell transformation in the setting of a predefined genetic lesion. While *Wnt-1* and *int-2* appear to be the dominant targets for MMTV, analysis of proviral insertions in the context of specific tumor precursor lesions may permit identification of additional target genes implicated in the stepwise progression of mammary neoplasia.

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1. Peters, G., Brookes, S., Smith, R. & Dickson, C. (1983) *Cell* **33,** 369–377.
2. Peters, G., Lee, A. E. & Dickson, C. (1984) *Nature (London)* **309,** 273–275.
3. Morris, D. W., Barry, P. A., Bradshaw, H. D., Jr., & Cardiff, R. D. (1990) *J. Virol.* **64,** 1794–1802.
4. Jakobovits, A., Shackleford, G. M., Varmus, H. E. & Martin, G. R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7806–7810.
5. Wilkinson, D. G., Bhatt, S. & McMahon, A. P. (1989) *Development (Cambridge, U.K.)* **105,** 131–136.
6. Mansour, S. L., Goddard, J. M. & Capecchi, M. R. (1993) *Development (Cambridge, U.K.)* **117,** 13–28.
7. Muller, W. J., Lee, F. S., Dickson, C., Peters, G., Pattengale, P. & Leder, P. (1990) *EMBO J.* **9,** 907–913.
8. Stamp, G., Fantl, V., Poulsom, R., Jamieson, S., Smith, R., Peters, G. & Dickson, C. (1992) *Cell Growth Differ.* **3,** 929–938.
9. Ornitz, D. M., Moreadith, R. W. & Leder, P. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 698–702.
10. Lee, F. S. (1993) Ph.D. dissertation (Harvard Univ., Cambridge, MA).
11. Shackleford, G. M., MacArthur, C. A., Kwan, H. C. & Varmus, H. E. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 740–744.
12. Nusse, R. & Varmus, H. E. (1982) *Cell* **31,** 99–109.
13. Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigel, D. & Nusse, R. (1987) *Cell* **50,** 649–657.
14. Peters, G., Lee, A. E. & Dickson, C. (1986) *Nature (London)* **320,** 628–631.
15. Kwan, H., Pecenka, V., Tsukamoto, A., Parslow, T. G., Guzman, R., Lin, T.-P., Muller, W. J., Lee, F. S., Leder, P. & Varmus, H. E. (1992) *Mol. Cell. Biol.* **12,** 147–154.
16. Shackleford, G. M. & Varmus, H. E. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9655–9659.
17. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
18. Fung, Y. K. T., Shackleford, G. M., Brown, A. M. C., Sanders, G. S. & Varmus, H. E. (1985) *Mol. Cell. Biol.* **5,** 3337–3344.
19. Dixon, M., Deed, R., Acland, P., Moore, R., Whyte, A., Peters, G. & Dickson, C. (1989) *Mol. Cell. Biol.* **9,** 4896–4902.
20. Gallahan, D. & Callahan, R. (1987) *J. Virol.* **61,** 66–74.
21. Roelink, H., Wagenaar, E., Lopes Da Silva, S. & Nusse, R. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4519–4523.
22. Dunn, T. B. (1959) in *Physiopathology of Cancer*, ed. Homburger, F. (Hoeber, New York), pp. 38–84.
23. Moore, R., Dixon, M., Smith, R., Peters, G. & Dickson, C. (1987) *J. Virol.* **61,** 480–490.
24. Frankel, W. N., Stoye, J. P., Taylor, B. A. & Coffin, J. M. (1989) *J. Virol.* **63,** 3810–3821.
25. Adamson, M. C., Dennis, C., Delaney, S., Christiansen, J., Monkley, S., Kozak, C. A. & Wainwright, B. (1994) *Genomics* **24,** 9–13.
26. Kelly, G. M., Lai, C. J. & Moon, R. T. (1993) *Dev. Biol.* **158,** 113–121.
27. Wolda, S. L. & Moon, R. T. (1992) *Oncogene* **7,** 1941–1947.
28. Sidow, A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5098–5102.
29. Marchetti, A., Robbins, J., Campbell, G., Buttitta, F., Squartini, F., Bistocchi, M. & Callahan, R. (1991) *J. Virol.* **65,** 4550–4554.
30. Clausse, N., Smith, R., Calberg-Baacq, C.-M., Peters, G. & Dickson, C. (1993) *Int. J. Cancer* **55,** 157–163.
31. Christian, J. L., Olsen, D. J. & Moon, R. T. (1992) *EMBO J.* **11,** 33–41.