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Molecular Cloning and Chromosomal Assignment of the Human Homolog of *int*-1, a Mouse Gene Implicated in Mammary Tumorigenesis

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Viral mammary tumorigenesis in mice is frequently initiated by proviral activation of a highly conserved cellular gene called *int*-1. We have cloned the human homolog of this putative mammary oncogene and compared its structure to that of the mouse gene by heteroduplex analysis. The human *int*-1 gene was localized on chromosome 12 by use of somatic cell hybrids.

The genetic events leading to breast cancer can be studied experimentally in mice infected with mouse mammary tumor virus (MMTV). This retrovirus induces adenocarcinomas of the mammary gland after a long latency period. MMTV does not transform cells in culture. From these biological properties, as well as from a biochemical analysis of the viral genome, it has been concluded that MMTV does not carry a viral oncogene (14).

The absence of a viral oncogene does not preclude the use of MMTV as a tool for studying oncogenes operating in mammary oncogenesis. Other slowly oncogenic retroviruses, such as avian leukosis virus, activate, by insertional mutagenesis, host cell oncogenes (2, 8). These cellular genes, c-*myc* in bursal lymphomas (2, 8) and c-*erb* (1) in erythroleukemias, were previously identified as cellular progenitors of viral oncogenes of acutely transforming retroviruses. In mammary tumors induced by MMTV, previously unknown genes, called *int*-1 (7) and *int*-2 (9), were implicated as cellular oncogenes activated by proviral insertion. These genes were identified by "transposon tagging": MMTV proviral DNA was used as a probe to clone host cell DNA adjacent to a provirus from a mammary tumor bearing a single integrated provirus. The cellular domains appeared to be occupied by MMTV proviruses in a large number of independent mammary tumors and were actively transcribed as a consequence of proviral integration (6).

Probes from the transcriptional domain of *int*-1 detect, at a reduced hybridization stringency, homologous sequences in various other organisms, including humans and *Drosophila* spp. (6). We have used this high degree of conservation to isolate a molecular clone of the human *int*-1 homolog.

In initial experiments, the human *int*-1 homolog was characterized by digestion of DNA from normal human tissues with restriction enzymes, followed by Southern blotting (13) and hybridization with mouse *int*-1 probes. Only a single copy of *int*-1 was found in the human genome.

The human *int*-1 was isolated from a library of human placental DNA constructed in the bacteriophage vector Charon 4A. From 5×10^5 individual recombinant phages we isolated two positive phage clones by hybridization with a mouse *int*-1 probe under moderately stringent conditions (6). Restriction enzyme analysis proved that the clones were partially overlapping and that they both corresponded to the authentic human *int*-1 gene.

The extent of homology between the mouse and human *int*-1 sequences was determined by examination of heteroduplex structures with an electron microscope. For this purpose, we selected a fragment from the mouse *int*-1 locus that contained the transcriptional unit of the gene. From S1 nuclease analysis of *int*-1 RNA in mouse mammary tumors, we established (15) that the transcriptional unit consists of at least four exons all present on a 12-kilobase (kb) *Bgl*II fragment (Fig. 1B). The polyadenylation signal has been mapped to 130 nucleotides from the *Bgl*II site, but the transcription initiation point of *int*-1 has not been mapped with certainty. This fragment was denatured and annealed to a 13.2-kb *Eco*RI fragment from the human *int*-1 clone (Fig. 1A shows a restriction map of this fragment).

Examination of the molecules with the electron microscope revealed heteroduplex structures (Fig. 1C). A tracing of the molecules is shown in Fig. 1D.

A typical heteroduplex contained four regions of homology between the mouse gene and the human gene. The location and length of the double-stranded regions corresponded to the exon sequences of the mouse gene (Fig. 1B). In general, however, the length of the duplex regions exceeded the length of the exons, especially at the 5′ end of the gene, where duplex region I was considerably longer than exon I. We interpret this as homology in the 5′ regulatory sequences of the two genes, but as yet we know little about the *int*-1 promoter region. More upstream from region I no homology was observed; the two strands remained separated over a distance of 5 to 6 kb, with a peculiar hairpin structure in one of the molecules.

At the 3′ ends of the heteroduplex molecules, double-stranded region IV did not continue into two single strands. This discontinuity is apparently due to the close proximity of the end of the mouse *int*-1 gene to the boundary of the *Bgl*II fragment. The segment in between the *Bgl*II site and the polyadenylation site either is too short to visualize or is

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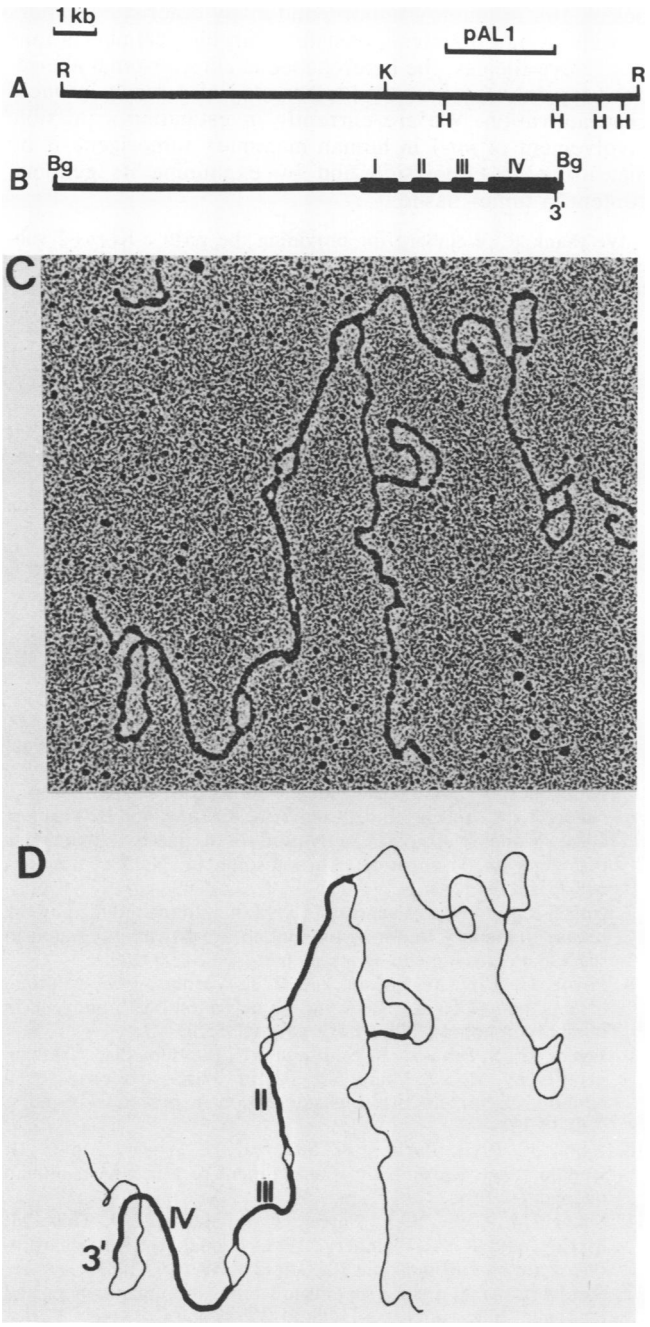

FIG. 1. Heteroduplex analysis of mouse and human *int*-1 DNA. (A) 13.2-kb *Eco*RI (R) fragment and some other restriction sites (*Hin*dIII [H]; *Kpn* [K]) of the human *int*-1 gene. The position of the pAL1 subclone that was used as a hybridization probe is also shown. (B) 12-kb *Bgl*II (Bg) fragment of the mouse *int*-1 gene. The positions of the exons (see reference 15) are indicated by thick bars. (C) Electron micrograph of a typical heteroduplex molecule. (D) Tracing of the molecule shown in (c). The thick lines correspond to double-stranded regions.

conserved between mice and humans. The protruding single strand belongs to the human *int*-1 DNA molecule.

This heteroduplex analysis illustrates the high extent of homology between the mouse and human *int*-1 genes and confirms the authenticity of the human *int*-1 clone. This

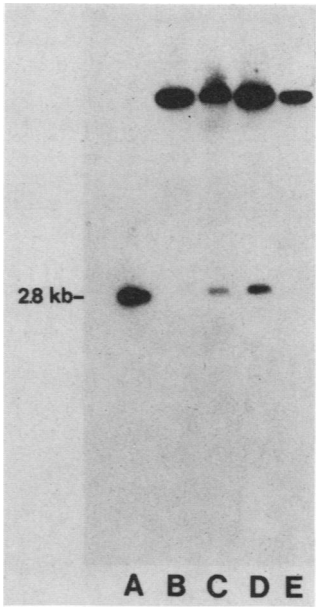

FIG. 2. Hybridization of the pAL1 probe to *Hin*dIII-digested human DNA (lane A), Chinese hamster DNA (lane B), and three different hybrid cell clones (lanes C through E). The 2.8-kb fragment is diagnostic for the human *int*-1 gene.

clone will be used to examine the structure and expression of *int*-1 in human mammary tumors and in other malignancies. As a first step in this direction, we have established the chromosomal location on *int*-1, in view of the recent accumulation of evidence that chromosomal aberrations may result in the activation of cellular oncogenes (for example, the chromosomal translocations in B-cell tumors affecting

TABLE 1. Segregation of *int*-1 with human chromosomes in human-rodent somatic cell hybrids[a]

Chromosome	No. of hybrids with indicated chromosome/*int*-1 fragment pattern			
	+/+	+/−	−/+	−/−
1	11	4	8	16
2	8	3	11	17
3	9	5	9	16
4	14	10	5	10
5	12	6	8	13
6	16	9	3	11
7	7	5	11	16
8	13	11	6	9
9	10	9	9	11
10	12	5	5	17
11	10	7	7	15
12	20	0	1	18
13	5	3	14	17
14	11	6	7	15
15	3	4	16	16
16	9	7	10	13
17	10	12	9	8
18	8	5	11	15
19	8	8	10	13
20	9	8	10	12
21	12	11	7	9
22	12	11	7	9

[a] A total of 39 clones were tested. Chromosome spreads were R-banded with acridine orange after heat denaturation. At least 16 metaphases were analyzed per hybrid cell line.

TABLE 2. Presence or absence of (12:21) (q14:q21) LE translocation chromosomes, the chromosome 12 markers PEPB, LDHB and c-Ki-*ras*-2, and human *int*-1 sequences in two somatic cell hybrids

Hybrid clone	Presence (+) or absence (−) of:							
	Human chromosome				Chromosome 12 marker[a]			*int*-1
	12	21	12:21	21:12	PEPB	LDHB	c-Ki-*ras*-2	
A1WCB	−	+	−	+	+	−	−	−
A1WBF2	−	+	+	−	−	+	+	+

[a] LDHB and PEPB isoenzymes were analyzed by cellulose acetate electrophoresis. The c-Ki-*ras*-2 oncogene was analyzed by molecular hybridization with the p640 probe (11).

the c-*myc* gene [4, 16]). The mode of proviral activation of *int*-1 in mammary tumors is very reminiscent of the activation of c-*myc* in avian leukosis virus-induced chicken bursal lymphomas (2, 6, 8); accordingly, *int*-1 may be activated by chromosomal translocations as well. An assignment to a particular human chromosome could be helpful in investigating this possibility as well as in providing a DNA marker specific for a human chromosome.

A panel of somatic cell hybrids between human cells and rodent cells segregating human chromosomes was examined for the presence of *int*-1. DNA from the hybrids was digested with several restriction enzymes and blot hybridized to the pAL1 probe, a 2.8-kb *Hin*dIII fragment of *int*-1 (see Fig. 1A), chosen because it does not contain highly repetitive human sequences. By selecting an appropriate enzyme, *Hin*dIII, we could differentiate the human sequences from the rodent *int*-1 homolog, which even under highly stringent hybridization conditions were still prominent in the hybrid cell DNA as a 12-kb band (Fig. 2). The presence of human *int*-1 restriction fragments in the hybrid cells was correlated with the chromosome content of the hybrid.

Table 1 shows that the human *int*-1 restriction fragments segregated almost completely with chromosome 12. In 39 hybrids, one discordancy was found: a hybrid clone with detectable *int*-1 sequences but no recognizable chromosome 12. Chromosome breakage probably resulted in the loss of fragments of chromosome 12, with retention of *int*-1 containing fragments. Discordancies with other chromosomes were invariably higher, allowing the conclusion that *int*-1 is present on human chromosome 12. A more specific assignment was made possible by annealing of the *int*-1 probe to hybrid cells containing (12:21) (q14:q21) LE translocations of chromosome 12 (Table 2). Hybrid A1WBF2 contained the q14 to pter part of chromosome 12, translocated to a part of chromosome 21. This hybrid clone had retained the isoenzyme marker lactate dehydrogenase (LDHB) (located in chromosome 12, p 12.2) and the DNA marker c-Ki-*ras*-2 (located in chromosome 12, p 12.1 [3, 5, 10, 11]) and also contained *int*-1 sequences. Hybrid A1WCB contained the reverse complement of the same chromosomal translocation and was positive for the isoenzyme marker peptidase (PEPB) but negative for *int*-1, LDHB, and c-Ki-*ras*-2. Thus, *int*-1 is located on the q14 to pter part of chromosome 12.

The chromosomal assignment of *int*-1 does not point immediately to a possible involvement in human cancers. Chromosome 12 is not frequently found to be translocated or otherwise altered in those human malignancies, such as leukemias, that contain well-defined karyological abnormalities (4, 16). Mammary tumors, and many other solid tumors as well, do not manifest consistent karyological aberrations (12). Nevertheless, the heterogeneous chromosomal aberrations typical of mammary tumors may also result in oncogene activation. We are currently investigating a possible involvement of *int*-1 in human mammary tumorigenesis by measuring its expression and by examining its genomic content in tumor tissue.

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