The nucleotide sequence of the human *int*-1 mammary oncogene; evolutionary conservation of coding and non-coding sequences

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The mouse mammary tumor virus can induce mammary tumors in mice by proviral activation of an evolutionarily conserved cellular oncogene called *int*-1. Here we present the nucleotide sequence of the human homologue of *int*-1, and compare it with the mouse gene. Like the mouse gene, the human homologue contains a reading frame of 370 amino acids, with only four substitutions. The amino acid changes are all in the hydrophobic leader domain of the *int*-1 encoded protein, and do not significantly alter its hydropathic index. The conservation between the mouse and the human *int*-1 genes is not restricted to exons; extensive parts of the introns are also homologous. Thus, *int*-1 ranks among the most conserved genes known, a property shared with other oncogenes. *Key words:* conservation/human oncogene/nucleotide sequence

Introduction

Mammary tumors in mice infected with the mouse mammary tumor virus (MMTV) offer an experimental route to the identification of cellular oncogenes: by molecular cloning of host-cell DNA adjacent to integrated MMTV proviral elements (Varmus, 1984). This approach has led to the discovery of two different genes, *int*-1 and *int*-2, that are often trancriptionally activated as a consequence of nearby proviral insertions in mammary tumors (Nusse and Varmus, 1982; Nusse *et al.*, 1984; Peters *et al.*, 1983). The proviral insertions at the *int*-1 locus strongly implicate expression of an intact gene product in tumorigenesis; many insertions were found in the transcriptional unit of the gene, but the protein-encoding domain is always left intact (Van Ooyen and Nusse, 1984). This protein is, as deduced from the nucleotide sequence, 370 amino acids long, and does not resemble any known gene product.

The *int*-1 gene is, as many cellular oncogenes are, conserved in evolution, with homologous sequences in organisms ranging from *Drosophila* to man (Nusse *et al.*, 1984). We have cloned the human homologue of *int*-1, and assigned it to chromosome 12 (Van 't Veer *et al.*, 1984). The homology between the mouse and the human *int*-1 DNA was illustrated by a heteroduplex analysis. In this paper, we present the complete nucleotide sequence of the human *int*-1 gene and compare it with that of the mouse gene. Extensive homologies are found, both in the proteinencoding domain and in some intron areas.

Results and Discussion

Sequence analysis of the human int-1 gene

The human int-1 gene has been cloned as part of a 13.2-kb EcoRI

fragment from a bacteriophage library of human placental DNA. The approximate position of the gene on this DNA fragment was first determined by the heteroduplex analysis presented before (Van 't Veer *et al.*, 1984), and subsequently by restriction enzyme sites which were conserved between the human and mouse genes. The precise position of the gene was obtained from the nucleotide sequence of the homologous area. The sequencing strategy and the resulting structure of the human *int*-1 gene (see below) is shown in Figure 1. Arrows indicate the direction and extent of sequencing. All protein-encoding sequences and most of the non-coding parts were determined from both strands.

Comparison of the human and mouse int-1 sequences and derivation of the structure of the human gene

In Figure 2 we have aligned the nucleotide sequence of the mouse and human *int*-1 genes. The following strategy for lining up both sequences was used. First, the human gene was compared with the mouse sequence in blocks of 50 nucleotides, with the aid of a computer program. Subsequently, regions of homology of 60%or more were lined up according to previously published rules, to obtain maximal homology (Van Ooyen *et al.*, 1979). Deletions and insertions were introducd in the sequence of the mouse gene if necessary. Figure 2 presents the result of this comparison; the human *int*-1 gene is given as a continuous sequence.

The structure of the human gene was derived by comparison with the previously established exon-intron structure of the mouse homologue (Van Ooyen and Nusse, 1984). This structure is based on extensive S1 mapping data and on unpublished cDNA cloning experiments (H.E.Varmus, personal communication). The mouse gene consists of four exons, the last of which contains a long untranslated trailer region and a polyadenylation signal. The first exon that has been detected contains the translational start signal and a non-translated leader. This exon is preceded by a TATA box which is conserved, leading us to conclude tentatively that this signal indicates the authentic start of the *int*-1 gene, but it cannot be excluded that transcription starts further upstream. Possible upstream exons must be non-coding, because the ATG



Fig. 1. Sequencing strategy and structure of the human *int*-1 gene. Arrows indicate direction and extent of reading of the sequenced fragments. Arrows in the upper part represent (+) strand sequences and in the lower part (-) strand sequences. The structure of the gene is derived from a comparison with the corresponding mouse gene (Figure 2); blocks represent exons; coding sequences are black; K: *Kpn*I; H: *Hind*III.

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CAGCTGAGTGAGGCGGGCGCGCGTGGGAGGGTGTCCCCAAGGGGAGGGGTCCGCGGGCCAGTGCAGGCCCGGAGGCGGGGCCACCGGGCGGG	120
GTCAGCTCTCGGCTCAGACGGGCGGGAACCACAGCCCCGCTCGCT	240
TATA box start? TTCAGCCAGCGCCGCCACTATAAGAGGCGGTGCCGCCGCGGGGGCCGCGCCACCAGCCGGGGACCGCGAGCCATGCTGTCCGCCGCCGCCCCCAGGGTTGTTAAAGCCAGACTG -GTC-TGTACAC-TGACAC-TGACAC-TGACAC-T	360
Met Gly Leu Trp CGAACTCTCGCCACTGCCGCCACCGCGCGCGCGCCACCACCGCGGGCAACAACCAAAGTCGCCGCAACTGCAGCAGAGCGGGCAAAGCCAGGCAGG	476
Ala Leu Leu Pro Gly Trp Val Ser Ala Thr Leu Leu Leu Ala Leu Ala Ala Leu Pro Ala Ala Leu Ala Ala Asn Ser Ser Gly Arg Trp GCG CTG TTG CCT GGC TGG GTT TCT GCT ACG CTG CTG CTG GCG CTG GCC GCT CTG CCC GCA GCC CTG GCT GCC AAC AGC AGT GGC CGA TGG C	566
Tr splice TG GTAAGTGAGCTGGTGCGGGGTCGCCACTTGTCCCGCGGCACAGAGCCAGGGGCCAACCCTACCCAGCTCCCACGCTCTGGGATCCGTCTGCCGACAGGCTCCCTCC	685
TCCCTCCGCGACACCGAAGGGCGATCTGGCATGAAACTGCCCCAGACTCCAGCTCTGTACAAGTGGGGCGAATGATCCGCCGGGAGGCCTAAGATACCCCAGGCAGG	805
CATCTAGCACCGCCCTTCCCCTTTGAGCGCCAACTCCAGCCTCACGGCGGTGGCTCACCACAGGTTTCCCCACCTCGGGAAGTGAAGGGCCAGGAGTTCGCCTAGAAAGGAGGGGAGAAG	925
AGGGTGGGACTCCTAAGCATTTCACGCCTTGGGTGGGCAAGAACTGCAGGCCATGATTATCTCGCTCAGGCTGACCGGAAGAGGCTCGGAGATCCAAGGTAGACACTCGGTCTCCGGGTA 289 nucleotides	1045
CCTCCTCTGTCCAGTCTCCGGACCTAGGGGCTCAGGCGAGCAGCCCTGGGACTACTGGGCACACAAGTCTGGACGCCCAGTTCTTTCAAATTAGTGAGCCTGGGAGAGCGGGTATTATT	1165
splice p AATCTCCCGCCATTCTCCCAGCCACATACCCCCAGGAAGAGGACCGGGTGGCACAGTTTTTATGGTTAGGGTGCGGATCCCCTTCCTGAGCCTGAGCTATCATACGTCCCACCAG G TACAA-GC- <u>-CTG-</u> AA-CC-TA	1282
Gly Ile Val Asm Val Ala Ser Ser Thr Asm Leu Leu Thr Asp Ser Lys Ser Leu Gln Leu Val Leu Glu Pro Ser Leu Gln Leu Leu Ser GGT ATT GTG AAC GTA GCC TCC TCC ACG AAC CTG CTT ACA GAC TCC AAG AGT CTG CAA CTG GTA CTC GAG CCC AGT CTG CAG CTG TTG AGC CC A	1372
Arg Lys Gln Arg Arg Leu Ile Arg Gln Asn Pro Gly Ile Leu His Ser Val Ser Gly Gly Leu Gln Ser Ala Val Arg Glu Cys Lys Trp CGC AAA CAG CGG CGC CTG ATA CGC CAA AAT CCG GGG ATC CTG CAC AGC GTG AGT GGG GGG CTG CAG AGT GCC GTG CGC GAG TGC AAG TGG GACAGC	1462
GIN PHE Arg Asn Arg Arg Trp Asn Cys Pro Thr Ala Pro Gly Pro His Leu Phe Gly Lys Ile Val Asn Arg G splice CAG TTC CGG AAT CGC CGC TGG AAC TGT CCC ACT GCT CCA GGG CCC CAC CTC TTC GGC AAG ATC GTC AAC CGA G GTGGGTGCCCAGGAAGGCGACG AAC	1557
CTTCCGGGAGCAGGGGAAACGCGGGGTCACCCCCAGGGCATGGGCGGGC	1677
GCCAGCTCGGGGCCAGACTTCTACCAGGCGTTTTCCAGCCGTGCACCCTGGAAACGAAGCTTAACTTTTCTGAGCTACTGCCCCAGATAAAGAAAG	1797
CCGCCGCTTTCCCCCAGCCTCTCTCAAAAGCGCCTGGGAAGCTGCTCTCTGCAGGC3TGTGTCTGGCCTCTCGCCCAGCAAGGCTTGCACCGCCAAAATGGGCCGAAAGTTTTGGGCTGC -AT-TTTTCG-TAAAGTA- <u>GT</u> GTTG-AA-TT(CTAG	1917
GAAGAAGTCTTGGGGATGTATGGTTCTTCCGCTCCCCTCTTCGGTTTGTCTCTCTGGGGCTGCT	2037
TACGCCCGTGGACGTGGCTGCCTGCCTCACGCACCTGCTTTCTCTACTAGCCCTAGAGACCAGCTTTCCAGCACTGCCGGCCCTGGTCCTCAGGACTCAAAGTGCGGAGTCGGGGGTGGGA	2157
splice ly Cys Arg Glu Thr Ala Phe Ile Phe Ala TTCCGGTCCCAAGCCCTTCATGAGGGTGCTGGCCGCGCGCCCCGCGTACCCCCTGGTGATCCCCGCTCCCTTCTCCCCACAG GC TGT CGA GAA ACG GCG TTT ATC TTC GCT 	2266
Ile Thr Ser Ala Gly Val Thr His Ser Val Ala Arg Ser Cys Ser Glu Gly Ser Ile Glu Ser Cys Thr Cys Asp Tyr Arg Arg Arg Gly ATC ACC TCC GCC GGG GTC ACC CAT TCG GTG GCG CGC TCC TGC TCA GAA GGT TCC ATC GAA TCC TGC ACG TGT GAC TAC CGG CGG CGC GGC 	2356
Pro Gly Gly Pro Asp Trp His Trp Gly Gly Cys Ser Asp Asn Ile Asp Phe Gly Arg Leu Phe Gly Arg Glu Phe Val Asp Ser Gly Glu CCC GGG GGC CCC GAC TGG CAC TGG GGG GGC TGC AGC GAC AAC ATT GAC TTC GGC CGC CTC TTC GGC CGG GAG TTC GTG GAC TCC GGG GAGT	2446
Lys Gly Arg Asp Leu Arg Phe Leu Met Asn Leu His Asn Asn Glu Ala Gly Arg Thr splice AAG GGG CGG GAC CTG CGC TTC CTC ATG AAC CTT CAC AAC AAC GAG GCA GGC CGT ACG GTGAGCTTTGAGAGGGCTCCGCACCCTAAGCGGAGCGG	2547
CAACCTCGGGCTGGGGAAGTGACGGTCGGTGAGATAAGGCAAGGGGCACCAGGAGAGGGGCGTCCTGGGAGAGCCGGAGGCTTGGAACGAAGACGGAGAATAGAGGAGACAGTGGCTGAGG 230 nucleotides	2667
GCAAAGGTATGTCTGGCCCGCGGACAGGTAGAAGAGGTTGCAAATCAAGCACAGTCTCTTCGCTGTACAGATTCGAAAAATAAGCCTGAGAGGCCGAGACTGACT	2787
GGGTTGGGCAGGGTTTCCAAATCTCAGCGGAACATTTCGCGCCTCCCTTCCCCTGGGCTCAGCTAGGCCTGGGCCTTTGCTGAGGTCCGGCCCCCGTGGCGTCCGGGAGAGGGCAGTGTC -ACTC-AA-GCTGCGCTA-TTC-C-ATTC-CAAGA(97 nucleotides	2907

splice Thr Val Phe Ser Glu Met Arg Gln Glu Cys Lys Cys His Gly Met TGGGAGGGTGACTCTGGCCCGGTGCCCTGGGACACTCTTTCTT)10
Ser Gly Ser Cys Thr Val Arg Thr Cys Trp Met Arg Leu Pro Thr Leu Arg Ala Val Gly Asp Val Leu Arg Asp Arg Phe Asp Gly Ala TCC GGC TCA TGC ACG GTG CGC-ACG TGC TGG ATG CGG CTG CCC ACG CTG CGC GCC GTG GGC GAT GTG CTG CGC GAC CGC TTC GAC GGC GCC 31 C	00
Ser Arg Val Leu Tyr Gly Asn Arg Gly Ser Asn Arg Ala Ser Arg Ala Glu Leu Leu Arg Leu Glu Pro Glu Asp Pro Ala His Lys Pro TCG CGC GTC CTG TAC GGC AAC CGC GGC AGC AAC CGC GCT TCG CGA GCG GAG CTG CTG CGC CTG GAG CCG GAA GAC CCG GCC CAC AAA CCG CTTA	190
Pro Ser Pro His Asp Leu Val Tyr Phe Glu Lys Ser Pro Asn Phe Cys Thr Tyr Ser Gly Arg Leu Gly Thr Ala Gly Thr Ala Gly Arg CCC TCC CCC CAC GAC CTC GTC TAC TTC GAG AAA TCG CCC AAC TTC TGC ACG TAC AGC GGA CGC CTG GGC ACA GCA GGC ACG GCA GGG CGC 32 	280
Ala Cys Asn Ser Ser Pro Ala Leu Asp Gly Cys Glu Leu Leu Cys Cys Gly Arg Gly His Arg Thr Arg Thr Gln Arg Val Thr Glu GCC TGT AAC AGC TCG TCG CCC GCG CTG GAC GGC TGC GAG CTG CTC TGC TGC GGC AGG GGC CAC CGC ACG CGC ACG CGC GTC ACC GAG 33 TCTT	370
Arg Cys Asn Cys Thr Phe His Trp Cys Cys His Val Ser Cys Arg Asn Cys Thr His Thr Arg Val Leu His Glu Cys Leu *** CGC TGC AAC TGC ACC TTC CAC TGG TGC TGC CAC GTC AGC TGC CGC AAC TGC ACG CAC ACG CGC GTA CTG CAC GAG TGT CTG TGA GGCGCTGCGC 34	462
GGACTCGCCCCCAGGAACGCTCTCCTCGAGCCCTCCCCCAAACAGACTCGCTAGCACTCAAGACCCGGTTATTCGCCCACCCGAGTACCTCCAGTCACACTCCCCGCGGTTCATACGCAT 35 CTC- -GGAA-GTCTT -GCG-TCG-T- -TT-CCG-GCG-T- 35	584
CCCATCTCTCCCACTTCCTCCTACCTGGGGACTCCTCAAACCACTTGCCTGGGGCGGCATGAACCCTCTTGCCATCCTGATGGACCTGCCCCGGACCTAACCTCCCTC	704
AGACCCCTTGTTGCACTGCCCCCTGCTTGGCCAGGAGGTGAGAGGAGGAGGAGGAGGGTCGGGTCCCCCCGGGGGCCGGCTCCTGATGGTGTCATTCTGCCTGC	324
T CTGCCTCTTCTTCCCCCTTGTCCTGCGTTTTCTCCCGGGTCCTCC	3 44
CCACCTGTAGCTGAAGCAGGAGGTTACAGGGCAAAAGGGCAGCTGTGATGATGTGGGGAATGAGGTTGGGGGGAACCAGCAGAAATGCCCCCCATTCTCCCAGTCTCTGTCGTGGAGCCATTG 40 CTC-GA-TTGCGGAAAGCT-A <u>A</u> CTCTCAAT <u>-</u> TTC-TCC-TCC-TC	064
AACAGCTGTGAGCCATGCCTCCCTGGGCCACCTCCTACCCCTTCCTGTCCTGCCTCCTCATCAGTGTGTAAATAATTTGCACTGAAACGTGGATACAGAGCCACGAGTTTGGATGTTGTA 41	184
AATAAAACTATTTATTGTGCTGGGTCCCAGCCTGGTTTGCAAAGACCACCCCCAACCCAACCCCAATCCCTCTCCACTCTTCTCCCCTGCAGCCTTTTCTGGTCCCTCTTCTC 	304
polyadenylation signal TCCTCAGTTTCTCAAAGATGCGTTTGCCTCCTGGAATCAGTATTTCCTTCC	424
TTTATCGATGACTTGGTGGCTTTTCCTTGAATCCAGAACACAACCTTGTTTGT	522

Fig. 2. Sequence of the human *int*-1 gene and comparison with the mouse homologue. The plus strand sequence of the human gene is shown as a continuum (top line). The mouse gene is aligned by rules explained in the text. Only regions of high homology are shown. Identical nucleotides are indicated by dashes; extra nucleotides are indicated in the mouse sequences; empty spots represent gaps. Regions of low homology are between brackets; the number of nucleotides in the corresponding area of the mouse sequence is shown. The amino acid sequence of the human gene, as well as splice sites, TATA box and polyadenylation signal are derived by comparison with the mouse gene. Amino acids which are different in both genes are boxed. Cysteines are indicated by dots. Variants of the conserved sequence CTGAC involved in lariat formation during splicing are underlined.

start codon is preceded by stop codons in all three reading frames (Van Ooyen and Nusse, 1984).

The comparison of the mouse with the human *int*-1 gene shows conservation of all splice sites and transcriptional-regulation sequences (TATA box and polyadenylation signal). The derived intron-exon structure of the human gene is shown diagramatically in Figure 1.

The human int-1 protein

The human *int*-1 protein, as deduced from the nucleotide sequence, is very similar to the mouse protein. Both proteins are 370 amino acids long, with only four differences (boxed in Figure 2). These substitutions are found near the amino terminus of the protein, which is markedly hydrophobic. A hydropathicity profile of the human *int*-1 protein is presented in Figure 3, showing a typical hydrophobic leader domain. The four amino acid differences from the mouse gene are within this leader, but do not influence the hydropathicity profile. Thus, if the hydrophobic leader is cleaved off during transport, the entire active domain

of the *int*-1 protein is evolutionarily conserved between mouse and man. This extraordinary degree of sequence constraint indicates how essential the structure of the *int*-1 product is in determining its function.

Nevertheless, the normal function of *int*-1 remains enigmatic. The gene has been found to be expressed only in mouse mammary tumors bearing a proviral integration near the transcriptional unit, and not in any normal tissue tested so far. As reported before for the mouse *int*-1 protein, we have not found any similarity to other gene products (Van Ooyen and Nusse, 1984). A salient feature of the *int*-1 protein is the high content of cysteine residues, often in pairs, near the COOH terminus, as indicated by dots in Figure 2. Properties such as a hydrophobic leader and cysteine-rich domains have been found in receptor molecules, for example the epidermal growth factor, the low density lipoprotein and the insulin receptors, but the *int*-1 protein lacks the typical transmembrane domain of these receptors (Ullrich *et al.*, 1984; Ebina *et al.*, 1985). Alternative-



Fig. 3. Hydropathicity profile of *int*-1. Hydropathicity values for amino acids were taken from Hopp and Woods (1981). At every position the hexapeptide value was taken.



Fig. 4. Overall homology between human and mouse *int*-1. The human and mouse genes were compared in blocks of 50 nucleotides with the aid of a computer, without introduction of gaps in either of the sequences. Regions with >50% homology are shown. Exons are indicated by blocks, coding sequences are black.

ly, we speculate that the mature form of the *int*-1 protein is a growth factor. These molecules are often cysteine-rich and synthesized as precursors with hydrophobic leaders (cf. Feramisco *et al.*, 1985).

Conservation of non-coding sequences

The sequence comparison in Figure 2 shows that the homology between the mouse and the human gene is not restricted to the coding sequences. Besides homologies in the non-coding part of the mRNA sequence, extensive parts of the *int*-1 introns are highly conserved, most notably near the 5' end of the first and the second intron, and near the 3' end of the first intron. This homology extends far beyond what is generally found at intron boundaries (Breathnach and Chambon, 1981). A less pronounced, but significant homology is found in the center of all three introns. A diagram of the overall homology is shown in Figure 4.

The significance of these conservations is not clear, but explanations that come to mind are functions in regulation of gene expression or in splicing. A variant of the consensus sequence CTGAC involved in lariat formation during splicing (Keller and Noon, 1984) is found in all three introns (underlined in Figure 2) at 19-46 nucleotides from the 3' splice site. These sequences are within conserved areas, with the exception of the TTGAC sequence in the third intron, which is in a region of low overall homology.

Conserved areas in introns have also pointed to the existence of elements regulating specific gene expression, or enhancers, in immunoglobulin genes (Emorine *et al.*, 1983; Gillies *et al.*, 1983; Banerji *et al.*, 1983; Queen and Baltimore, 1983). Other genes, globin and thymidine kinase, for example, also carry intragenic regulatory elements (Wright *et al.*, 1984; Merrill *et al.*, 1984). The large area of homology upstream from the first exon could also serve a function in regulating gene expression, as it may contain the *int-*1 promoter.

None of the homologous areas outside the *int*-1 exons can encode proteins of substantial length since stop codons occur frequently.

Implications

The sequence comparison between the mouse and the human *int*-1 homologues presented here illustrates the evolutionary conservation that is characteristic for oncogenes (Bishop, 1983); and is even unprecedented within the oncogene family. Compared with those oncogenes for which both the murine and the human cellular homologues have been sequenced, the overall amino acid sequence homology of *int*-1 (99%) is higher than that of c-*myc* (93%, Bernard *et al.*, 1983), of c-*fos* (90%, Verma *et al.*, 1984) and of c-*mos* (75%, Blair *et al.*, 1984), of p53 (78%, Zakut-Hori *et al.*, 1985) and of c-Ki-*ras* (97%, George *et al.*, 1985).

The mouse *int-*1 gene can be activated by proviral insertions of MMTV, leading to some step in mammary carcinogenesis. Whether abnormal expression of the virtually identical human homologue can contribute to mammary tumorigenesis in man, in which no manifest replication of MMTV-like viruses has been observed, remains to be seen. Rather than by proviral insertion, activation of the gene might well occur by gene amplification, the hallmarks of which – double minute chromosomes – are often observed in human mammary tumors (Barker and Hsu, 1979; Gebhardt *et al.*, 1984).

Materials and methods

DNA sequence analysis

DNA sequencing was carried out according to the method of Maxam and Gilbert (1980). Labeling of 5' ends was carried out by treatment of restriction sites with calf intestine alkaline phosphatase (Boehringer) and phosphorylation with $[\gamma^{-32}P]ATP$ (Amersham) and T4 polynucleotide kinase (Boehringer). Restriction fragments were labeled at 3' ends with the large fragment of DNA polymerase I (New England Biolabs) and all four deoxynucleotides. Unlabeled nucleotides were at 20 μ M and the labeled [$\alpha^{-32}P$]dNTP at 1.5 μ M.

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