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Sequences coding for part of oncogene-induced transin are highly conserved in a related rat gene

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ABSTRACT

The transin gene is induced by oncogenes and epidermal growth factor (EGF). We report here the isolation of a related gene (transin-2 gene). The structures of these genes are very similar. Indeed, a stretch of 428 nucleotides of the transin gene containing both exon and intron sequences is 98% conserved in the transin-2 gene. However, the putative promoter regions of the genes show little sequence homology, apart from a short element related to a sequence involved in control of transcription by cyclic AMP or a tumour promoter. Expression of the transin-2 gene, unlike that of the transin gene, is not induced by EGF, dibutyryl cyclic AMP or cytochalasin D. Nevertheless, transin-2 RNA is expressed in several transformed rat embryo fibroblast cell lines, and can be induced by a tumour promoter. The proteins transin and transin-2 are ∿ 71% homologous in sequence. Both proteins show significant sequence homology with two connective tissue degrading metalloproteases. These homologies raise the possibility that expression of transin and transin-2 in transformed cells might play a role in tumour invasion.

INTRODUCTION

A number of oncogenes have been isolated and characterised to date (1, 2). Several of these oncogenes are related to genes for growth factors or their receptors. For instance, the c-fms proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor colony stimulating factor CSF-1 (3), while the cellular homologue of the oncogene v-sis is a gene encoding platelet-derived growth factor(4, 5). Other proto-oncogene proteins are also clearly related to cellular proteins involved in signal transmission. Thus ras proteins show some homology to the family of G proteins involved in a variety of signal transduction events (6, 7). Yet other proto-oncogenes such as c-myc and c-fos are responsive to growth factor stimulation in a variety of cell types (8, 9, 10, 11). Proto-oncogenes clearly have the capacity to play a fundamental role in cellular growth control, and activated oncogenes to disrupt this control in cancers. Studying the mode of action of oncogenes may thus further understanding of the differences between normal and cancerous cells.

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It is possible that various effects of oncogene activation in cells are due to changes in the expression of cellular genes, leading to either the production of new proteins characteristic of the transformed state, or to the loss of proteins normally expressed. For example, it has been shown that adenovirus-12 transformed cells are oncogenic because they escape from T-cell immunity. This is brought about by reducing the expression of class I transplantation antigens (12). Reduced expression of fibronectin and collagen (13, 14) has also been reported in transformed cells. A number of other genes have been isolated whose level of expression is significantly elevated in transformed cells compared to their normal counterparts (15, 16, 17, 18, 19, 20, 21). Several of these genes are linked to repeated sequences, and their role in transformation is unclear. On the other hand, some transformation-induced genes code for proteases. A well studied example is provided by plasminogen activator (22). Another example is the major excreted protein (MEP) of transformed 3T3 cells, which is an activable acid-protease (23). We have described previously (18, 19) an oncogene and growth-factor induced mRNA (transin mRNA) which codes for a secreted protease (39). Identification of such oncogene-induced proteases is important, as proteases can facilitate tumour invasion (22).

We show here that a second gene (transin-2 gene) related to the transin gene exists in rat DNA, and that this gene is also expressed in transformed but not in normal rat cells. Rat transin and transin-2 show extensive sequence homologies with collagenase and stromelysin (G. Murphy and A. Docherty, personal communication), two proteases which degrade extracellular matrix components : it is thus possible that transin and transin-2 are similar proteases, in which case they might play a role in tumour invasion.

MATERIALS AND METHODS

Materials. Cytochalasin D and dibutyryl cyclic AMP (dibutyryl cAMP) were from Sigma Chemical Co, St Louis, Mo. EGF was obtained from Bethesda Research Laboratories. Transforming growth factor α (TGFα) was from Penninsula.

Cell culture. Cultured cells were maintained in Dulbecco modified Eagle medium containing 10% fetal calf serum and appropriate antibiotics a 37°C in a 95% air- 5% CO_2 atmosphere. For analysis of RNA levels, cells were washed once in phosphate-buffered saline shortly after reaching confluence (≤12h) and the medium replaced with serum-free medium overnight. RNA was then harvested from transformed cell lines by Nonidet P40 lysis as previously

described (18, 19). Otherwise EGF or TGFα (20 ng/ml), cytochalasin D (10 nM), or dibutyryl cAMP (0.5 mM) were added to the serum-free medium for 8 h (EGF and TGFα) or 24 h (dibutyryl cAMP and cytochalasin D) before RNA isolation. Cell lines : Rat-1, Rat-1 1.2, RTras, B-77, FR3T3, MTT4 and PyT21 are described in (18). SVWT-A1 (24), FR3T3-BPV3 (25) and BPV3-TDI (25) are described in the indicated references. WTRSV, FR3T3 cells transformed by Rous sarcoma virus, were provided by F. Cuzin.

DNA and RNA analysis. Analysis of DNA and RNA samples by gel electrophoresis, transfer to nitrocellulose and hybridisation with nick-translated probes was as described previously (18, 19). For RNA analysis, two nick-translated probes were used to distinguish between transin and transin-2 mRNAs. The transin specific probe was a BglII-EcoRI fragment of pTR1 (18) containing nucleotides 1128-1771 of transin cDNA (Fig. 2). The transin-2 specific probe was an EcoRI fragment containing nucleotides 1005-1708 of transin-2 cDNA (Fig. 2). Hybridisations of RNA transferred to nitrocellulose were carried out in the presence of 50% formamide - 5 X SSC (1 x SSC is 0.15 M NaCl plus 0.015 M sodium citrate) - 1 X Denhardt solution - 20 mM sodium phosphate - 0.1% sodium dodecyl sulphate - 50 µg of salmon sperm DNA per ml - 4% dextran sulphate at 42°C. Filters were washed in 0.1 X SSC - 0.1% sodium dodecyl sulphate at 50°C. Hybridisation of DNA transferred to nitrocellulose was carried out either as for RNA or as above except that 40% formamide was used and filters were washed in 2 X SSC - 0.1% sodium dodecyl sulphate at 50°C (low stringency conditions). Probes used were a full-length transin cDNA (18) or a 525 base pair PstI-KpnI fragment thereof (nucleotides 155-680, Fig. 2).

Isolation of transin-2 gene and cDNA. PyT21 cell DNA was isolated as described by Maniatis et al. (26) and used to make a rat genomic DNA library in the bacteriophage vector EMBL3 (27) using standard techniques (26, 27). 10^6 recombinant phage were screened for sequences hybridising to a 525 base pair PstI-KpnI fragment of transin cDNA (nucleotides 155-680 of Fig. 2). EcoRI digests of DNA from 8 positive phage allowed them to be separated into 4 phage carrying inserts covering the transin gene and 4 carrying inserts covering the transin-2 gene using a transin gene map established previously (19). EcoRI fragments from the transin-2 gene were subcloned into pUN121 (28) to facilitate restriction enzyme mapping. Fragments carrying sequences hybridising to transin cDNA from pTR1 (18) were sequenced using the dideoxy technique (29) with buffer gradient gels (30) to identify the cross-hybridising zones. Vectors used were M13tg130 and M13tg131 (31). Sequences were

obtained either from restriction enzyme sites occurring in genomic DNA fragments (with the universal sequencing primer) or using chemically synthesised 17-mers for specific priming. The same strategies were used to sequence all the exons of the transin gene on genomic fragments isolated previously (19). B-77 RNA was isolated using the urea-LiCl technique (32). Double-stranded cDNA was made from B-77 poly(A)⁺RNA using a cDNA synthesis kit obtained from Amersham, and attached to EcoRI linkers before ligation to EcoRI digested λgt10, packaging, and infection of bacteria using standard procedures (26, 33). Recombinant phage carrying EcoRI inserts complementary to a transin-2 gene specific probe were identified by filter-hybridisation (26). EcoRI fragments from these phage were introduced into the EcoRI site of pUN121 (28). Resulting plasmids were used to prepare fragments for insertion into M13 vectors for sequencing using strategies outlined above. The transin-2 specific probe was an SstI-XhoI fragment of the transin-2 gene (see Fig. 1B, arrow SX).

RESULTS

A gene related to the transin gene.

We have described previously experiments investigating sequences hybridising to a full-length transin cDNA in Rat-1 cell genomic DNA (19). For the batch of DNA used, all hybridising fragments could be accounted for on the basis of one transin gene. We isolated this gene from the same batch of DNA and described its structure as determined by electron microscopy studies of transin gene -cDNA hybrids (19). During the course of further work we examined the transin gene structure in a number of other rat cell lines and in various rat tissues. In these samples we observed an additional EcoRI fragment of 7.9 kb which hybridised under the same conditions we had used before to transin cDNA and to a 525 bp PstI-KpnI fragment (nucleotides 155-680 in Fig. 2) of transin cDNA [Fig. 1A, starred band; compare lane 1 with Fig. 2 of (19)]. Subsequently we detected this additional fragment in DNA from other batches of Rat-1 cells. The reason why we did not observe this fragment in the original batch of Rat-1 DNA is not clear. Perphaps the corresponding Rat-1 cells had lost these sequences. Consistent with this interpretation, screening of a genomic library made from DNA from these Rat-1 cells led to isolation only of 4 transin (and no transin-2) genes. In any case, it seemed that in contrast to our original report, a second gene related to the transin gene existed in rat genomic DNA.

Rat genomic DNA complementary to the transin cDNA PstI-KpnI fragment

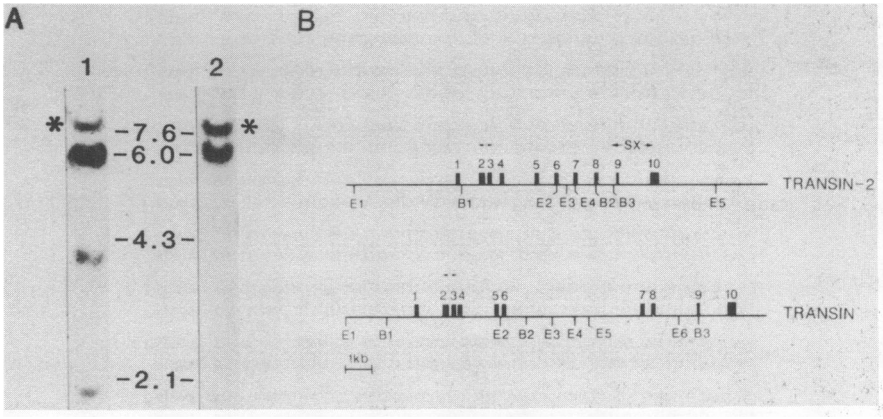

Fig. 1 : A. Southern analysis of EcoRI digested rat DNA. Lane 1, full size transin cDNA probe. Lane 2, 525 base pair PstI-KpnI fragment probe. Starred bands correspond to fragment E1-E2 of the transin-2 gene. In lane 1, remaining bands correspond in order of decreasing size to fragment E1-E2, a comigrating fragment from E6 to a downstream EcoRI site not shown, fragment E5-E6 and fragment E2-E3 of the transin gene as shown in B. Size markers are given in kilobase pairs. B. Maps of transin-2 and transin genes. Arrows show an area of conserved sequence discussed in the text. Arrow SX on the transin-2 gene map identifies an SstI-XhoI probe fragment. E = EcoRI; B= BamHI.

was obtained by screening a new library of 10^6 recombinant phage carrying ~15 kb fragments of rat cellular DNA (a batch of FR3T3 cell DNA which we had shown to contain the additional fragment) in the lambda phage vector EMBL3. Of the 8 phage which gave a positive signal with this probe, four contained regions of the transin gene we had isolated previously and four contained overlapping sequences apparently from a new gene. Three of these latter phage contained the 7.9 kb EcoRI fragment discussed above. One of the phage (φ18) contained a 5.5 kb EcoRI fragment which hybridised very weakly to transin cDNA under low stringency conditions. This fragment corresponded to a fragment observed under the same conditions on Southern blots of rat genomic DNA digested by EcoRI (data not shown; some additional very weak bands were observed on these blots under low stringency conditions, suggesting that yet other transin-related genes exist in the rat genome). Fragments of φ18 cross-hybridising with transin cDNA were sequenced. We compared the sequences obtained with the sequence of transin cDNA and that of a cloned cDNA corresponding to mRNA transcribed from the new gene (see below). This comparison allowed the determination of the structure of the new gene, which we propose to term the transin-2 gene (see Fig. 1B). Exon limits are indicated on the transin-2 cDNA sequence shown in Fig. 2.

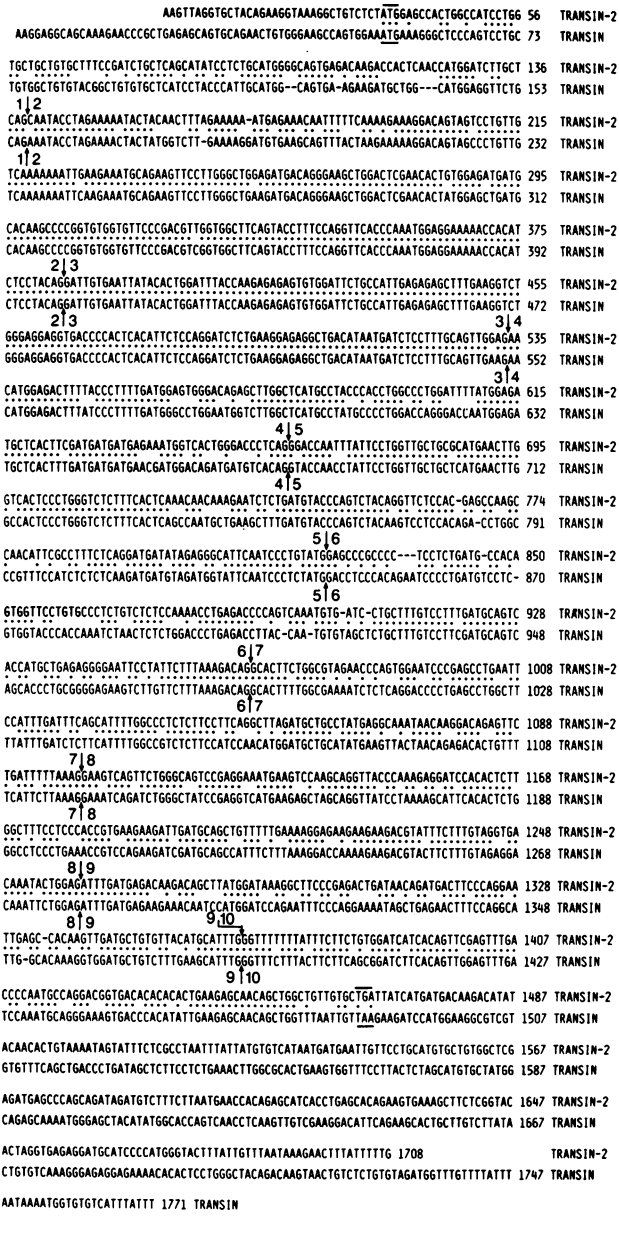

Fig. 2 : Comparison of the sequence of transin-2 cDNA to that of transin cDNA. Identical nucleotides are shown by dots. Dashes represent gaps introduced into sequences to improve alignment. No attempt was made to align 5'- or 3'-untranslated regions. Initiation and termination codons for protein synthesis are underlined (transin) or overlined (transin-2). Vertical arrows indicate exon limits, and exons are numbered as in Fig. 1B.

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AAGCTTTCTTCCATAATTTTGTTGAAAACATTTACTAGCCCTTCAGGTTAGGAGTCTTCATTCTCTTCTATACCTAATATCCTTAGGTTTGATCTTTTCATTGTGTCCTGGATTTCCAGA  120
TGTTTTGGGATAGCAGCTTTTTGGATTTTACATTCTCTTTGATGGTTGTGTCAATGTTTTCTATGGTACCTTCTGCCCCTGAGATCCTCTCTTCTATCTCTTGTGTTCTGTTGGTGATGC  240
TTGCATCTATGACTCCTGATCTCTTCCCTAGGTTTTCTATCTCCAGGGTTCTCTCCCCTTTGTGCTTTCTTTATTGTTTCTGTTTTCATTTTTAAATCCTGGACATTCATTTTTAAATTGT  360
TCAATTCCTTCACCCGTTTGGTTATGTTTTCCTATAATTCTTTAAGGGATTTTTTTGTTTCCTCTTTAAGAGCTTCTACTTGTTTACTTGTGTTGTCCTGCTTTTTTTTCCAAATATCTA  480
AATATTTTACAAACATTAATTACCCACTCAGTTCCTTTTCCTCTTTATAAACACAGAAGCCACATCCTTGAGATTAGGCATTCTTTCAAGTTTAGTAGGTTGAGAGATTCACTGATGTAA  600
GATATCCTCTTCATCCTATGAATGCACTTGCAGTCATTCAGATTAAAATTAGCACCCACATGAGTCATGCTTAGTGACCTAAACACCCTATAAAAGAGTAGCCGCTAAAGGGAAGATATG  720
GAAGTTAGGTGCTACAGAAGGTAAAGGCTGTCTCTATGGAGCCACTGGCCATCCTGGTGCTGCTGTGCTTTCCGATCTGCTCAGCATATCCTCTGCATGGGGCAGTGAGACAAGACCACT  840
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Fig. 3 : DNA sequence of the putative promoter region of the transin-2 gene. The TATA box sequence is marked with a wavy line. The sequence underlined by a straight line corresponds to part of exon 1 of the transin-2 gene. The cap site has been assigned as the nucleotide 30 bases distant from the TATAAA sequence element.

Our previous analysis of the transin gene structure relied in part on electron microscopy studies. In order to compare the structures of the transin and transin-2 genes in detail, we determined all the exon limits of the transin gene by sequencing the transin exons on cloned fragments of genomic DNA. The resulting transin gene structure is shown in Fig. 1B, and exon limits are indicated on the transin cDNA sequence shown in Fig. 2. This nucleotide sequence analysis allowed us to find two introns which had been too small to detect in the electron microscopy analysis. These introns lie within the region we termed exon 2 previously (19); their discovery has led to a renumbering of transin exons. (Exons 3-8 of Ref. 19 correspond to exons 5-10 of the gene map shown in Fig. 1. The genomic exons sequenced for both the transin and transin-2 genes include all of the mRNA coding information, ruling out the possibility of further undetected introns.

The transin and transin-2 genes have very similar structures. The most striking difference is the much larger size of the intron separating exons 6 and 7 of the transin gene compared to the size of the corresponding intron in the transin-2 gene. Much of this intron in the transin gene is taken up by a LINE repeat element [(34), data not shown]. Comparison of the transin and transin-2 cDNA sequences reveals that exon sequences of the two gene are related over much of their length (Fig. 2). However, transin gene sequences coding for the 5'- and 3'- untranslated regions of transin mRNA show very little homology to the corresponding sequences of the transin-2 gene. The putative promoter regions of the transin gene (19) and transin-2 genes (Fig. 3) also show little overall sequence homology. However, the transin and transin-2 genes have a conserved sequence of eight nucleotides 5'-ATGAGTCA-3' situated respectively 40 and 30 nucleotides upstream from their TATA boxes.

The transin and transin-2 cDNAs contain one exceptionally well

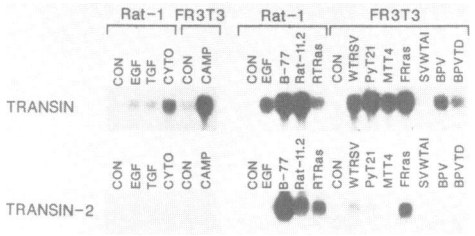

Fig. 4 : Expression of transin and transin-2 RNAs. 10 µg of cytoplasmic RNA was subjected to gel electrophoresis, transfer to nitrocellulose, and hybridization with transin (TRANSIN) or transin-2 (TRANSIN-2) specific probes as described in Materials and Methods. CON = untreated cells. EGF, TGF, CYTO, cAMP refer to cells treated with EGF, TGFα, cytochalasin D and dibutyryl cAMP, respectively. Transformed lines are as described in the text. BPV refers to line FR3T3-BPV3. BPVTD refers to line BPV3-TD1. SVWTAI are SV40 transformed FR3T3 cells. Identical samples of RNA were used for the two blots.

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                                  1↓2
MKGLPVLLWLCTAVCSSYPLHGS--EEDAGMEVLQKYLENYYGLEKDVKQFTKKKDSSPVVKKIQEMQKFLGLKMTGKLD 78  TRANSIN
MEPLAILVLLCFPICSAYPLHGAVRQDHSTMDLAQQYLEKYYNFRKNEKQFFKRKDSSPVVKKIEEMQKFLGLEMTGKLD     TRANSIN-2
MHSFPPLLLLLFWGVVSHSFPATLETQEQDVDLVQKYLEKYYNLKNDGRQVEKRRNSGPVVEKLKQMQEFFGLKVTGKPD     COLLAGENASE
                                      2↓3
SNTMELMHKPRCGVPDVGGFSTFPGSPKWRKNHISYRIVNYTLDLPRESVDSAIERALKVWEEVTPLTFSRISEGEADIM 158 TRANSIN
SNTVEMMHKPRCGVPDVGGFSTFPGSPKWRKNHISYRIVNYTLDLPRESVDSAIERALKVWEEVTPLTFSRISEGEADIM     TRANSIN-2
AETLKVMKQPRCGVPDVAQFVLTEGNPRWEQTHLRYRIENYTPDLPRADVDHAIEKAFQLWSNVTPLTFTKVSEGQADIM     COLLAGENASE
   3↓4                                    4↓5
ISFAVEEHGDFIPFDGPGMVLAHAYAPGPGTNGDAHFDDDERWTDDVTGTNLFLVAAHELGHSLGLFHSANAEALMYPVY 238 TRANSIN
ISFAVGEHGDFYPFDGVGQSLAHAYPPGPGFYGDAHFDDDEKWSLGPSGTNLFLVAAHELGHSLGLFHSNNKESLMYPVY     TRANSIN-2
ISFVRGDHRDNSPFDGPGGNLAHAFQPGPGIGGDAHFDEDERWTNNFREYNLHRVAAHELGHSLGLSHSTDIGALMYPSY     COLLAGENASE
                5↓6                                        6↓7
KSSTDLARFHLSQDDVDGIQSLYGPPTESPDVLVVPTKSNSLDPETLPMCSSALSFDAVSTLRGEVLFFKDRHFWRKSLR 318 TRANSIN
RFSTSQANIRLSQDDIEGIQSLYG-ARPSSDATVVPVPSVSPKPETPVKCDPALSFDAVTMLRGEFLFFKDRHFWRRTQW     TRANSIN-2
---TFSGDVQLAQDDIDGIQAIYG---------RSQNPVQPIGPQTPKACDSKLTFDAITTIRGEVMFFKDRFYMRTNPF     COLLAGENASE
                                  7↓8
TPEPGFYLISSFWPSLPSNMDAAYEVTNRDTVFILKGNQIWAIRGHEELAGYPKSIHT-LGLPETVQKIDAAISLKDQKK 397 TRANSIN
NPEPEFHLISAFWPSLPSGLDAAYEANNKDRVLIFKGSQFWAVRGNEVQAGYPKRIHT-LGFPPTVKKIDAAVFEKEKKK     TRANSIN-2
YPEVELNFISVFWPQLPNGLEAAYEFADRDEVRFFKGNKYWAVQGQNVLHGYPKDIYSSFGFPRTVKHIDAALSEENTGK     COLLAGENASE
      8↓9                                    9↓10
TYFFVEDKFWRFDEKKQSMDPEFPRKIAENFPGIGTKVDAVFEAFGFLYFFSGSSQLEFDPNAGKVTHILKSNSWFNC   475 TRANSIN
TYFFVGDKYWRFDETRQLMDKGFPRLITDDFPGIEPQVDAVLHAFGFFYFFCGSSQFEFDPNARTVTHTLKSNSWLLC     TRANSIN-2
TYFFVANKYWRYDEYKRSMDPSYPKMIAHDFPGIGHKVDAVFMKDGFFYFFHGTRQYKFDPKTKRILTLQKANSWFNCRKN     COLLAGENASE
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Fig. 5 : Comparison of protein sequences of transin, transin-2 and collagenase. Dots identify identical amino acids. Dashes indicate gaps introduced into sequences to improve alignments. Vertical arrows indicate the extents of coding regions of different exons in transin and transin-2 genes as shown in Fig. 1B.

conserved area, covering nucleotides 196-533 of the transin-2 cDNA and nucleotides 213-550 of transin cDNA (7 base changes between the two sequences). This area covers exon 3 and part of exon 2 of the two genes. The introns separating these exons in the two genes are also very homologous in sequence (2 base changes for an overall length of 91 nucleotides, data not shown). This highly conserved area is shown by arrows in Fig. 1B.

Expression of the transin-2 gene.

The transin-2 gene contains a 5.5 kb EcoRI fragment (E4 to E5, Fig. 1B) which hybridises weakly to transin cDNA under low stringency conditions. This fragment contains a 1.8 kb SstI-XhoI fragment (arrows SX in Fig. 1B) which is in part responsible for this hybridisation. We used this latter fragment to screen various cell lines for production of a transin-2 RNA. We detected this RNA as a 1.9 kb transcript in B-77 cells (Rous sarcoma virus-transformed Rat-1 cells). RNA from these cells was used to isolate a cloned transin-2 cDNA. Subsequently a larger range of cell lines was tested for production of transin and transin-2 RNAs using specific probes derived from the 3'-ends of the respective cloned cDNAs (see Materials and Methods).

The transin-specific probe detects transin RNA in rat cells treated with EGF, TGFα or cytochalasin D or, in the case of FR3T3 cells, with dibutyryl cAMP. None of these treatments gives rise to levels of RNA detectable with the transin-2 specific probe (Fig. 4). Both transin and transin-2 RNAs can be induced in Rat-1 cells by the phorbol ester TPA in the presence of some batches of serum [(19) and data not shown]. The transin RNA is detected in most of the transformed cell lines tested, namely cells (FR3T3 or Rat-1) transformed by Rous sarcoma virus (B-77, WTRSV), polyoma virus or polyoma virus middle T protein (Rat-1 1.2, PyT21, MTT4), a Ha-ras oncogene (RTras, FRras) or bovine papilloma virus type I (BPV, BPVTD). Transin-2 RNA is expressed in several of the above lines, but is not detected in FR3T3 cells transformed by bovine papilloma virus type I (BPV, BPVTD) or polyoma virus middle T protein (MTT4). Transin-2 RNA expression in transformed cells therefore does not parallel that of transin RNA.

Transin-2 protein sequence.

We compare in Fig. 5 the protein sequences of transin (18), transin-2 and a human fibroblast collagenase (35) [sequences deduced from the corresponding cDNA sequences]. Transin and transin-2 are ∿ 71% homologous in sequence, and indeed share a sequence of 78 amino-acids. Both transin and transin-2 are ∿ 49% homologous in sequence to the collagenase. The sequence of a human proteoglycanase stromelysin has recently been determined, and is

75% homologous to the rat transin sequence (G. Murphy and A. Docherty, personal communication). A comparison of the transin-2 and stromelysin sequences shows a homology of 70%.

DISCUSSION

Rat embryo fibroblast cell lines transformed by various oncogenes express a 1.9 kb mRNA, transin RNA. The transin gene is under complex control as it can also be induced by EGF, TGFα, a phorbol ester tumour promoter, cyclic AMP and cytochalasins (18,19). We show here that a second gene (transin-2 gene) related to the transin gene exists in the rat genome. The overall organisation of the two genes is similar. However, they are under different control in rat embryo fibroblasts, as neither EGF, TGFα, cyclic AMP nor cytochalasin D will induce expression of transin-2 RNA. Transin-2 RNA is however expressed in several transformed lines, though its expression does not always parallel that of transin RNA, and can, like transin RNA, be induced in the presence of some batches of serum by TPA.

The putative promoter regions of the transin and transin-2 genes show little overall sequence homology. However, both genes have a sequence 5'-ATGAGTCA-3' situated 30-40 nucleotides upstream from their TATA boxes which is related to a sequence (5'-TGACGTCA-3') believed to make up part of a cyclic AMP and phorbol ester-inducible DNA element (36, 37).

Transin-2 protein is 71% homologous in sequence to transin, and the two proteins share a stretch of 78 identical amino-acids. This extensive conserved sequence is a consequence of a high nucleotide sequence homology of the corresponding areas of the genomic genes. A stretch of 428 nucleotides of the transin gene covering part of exon 2, exon 3 and the intron separating them is found in an almost unchanged form (nine base changes) in the transin-2 gene. This conservation of both exon and intron sequence suggests that a recent gene conversion event may have taken place in this area.

Both transin and transin-2 are 48-49% homologous in sequence to a human fibroblast collagenase whose sequence has recently become available (35). Transin shows a much higher (75%) homology to (and may be the rat homologue of) a human protease stromelysin (G. Murphy and A. Docherty, personal communication). The principal substrates of stromelysin are proteoglycans and fibronectin (38). There is some data consistent with the hypothesis that transin is rat stromelysin. Thus we have shown that transin is a

secreted protein, and that immunopurified transin, purified from the culture medium of Rous sarcoma virus transformed Rat-1 cells using antibodies directed against a synthetic peptide corresponding to amino-acids 445-468 of transin, will digest casein (39). Fragments produced are similar to those obtained using purified stromelysin (38). Expression of rat transin cDNA in cos cells or CHO cells under control of the SV40 early gene promoter leads to the appearance in the culture medium of a casein degrading activity, which generates fragments similar to those observed with immunopurified transin. As is the case for stromelysin, this protease activity is latent and can be activated by p-aminophenyl mercuric acetate (39). Activated conditioned medium from CHO cells expressing rat transin is significantly more effective than conditioned medium from the parent CHO cells in solubilising an extracellular matrix laid down by human ombilical card endothelial vein cells, with preferential solubilisation of material labelled with ^{35}S-sulphate, presumably proteoglycans (R. Millon and R.B., unpublished observations). These data, taken together with the sequence homology noted above, strongly suggests that transin is rat stromelysin or a closely related protease.

The amino-acid sequence of transin-2 shown in Fig. 5 reveals a protein with hydrophobic N-terminal amino acids typical of a signal peptide. The homology of transin-2 to other proteases leads us to speculate that transin-2 is also a secreted protease, and that transin (stromelysin ?), transin-2 and collagenase make up a family of related connective tissue degrading metalloproteases, each perhaps with a different substrate specificity.

Oncogenes and growth factors induce expression of several protease genes. Thus collagenase can be induced in human fibroblasts by a variety of growth factors (40), and plasminogen activator levels are increased in many transformed cells (22). The major excreted protein (MEP), an acid activable protease, is also induced by both oncogenes and growth factors in mouse cells (17,23). Such proteases can be of importance in a number of situations where degradation of the extracellular matrix is required and growth factors or oncogenes intervene. Possible examples are tissue remodelling, tumor invasion and invasion of cells during development. The availability of transin and transin-2 genes should allow the role played by the corresponding proteins in some of these important biological processes to be assessed.

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