Two Alternatively Spliced Mouse Urokinase Receptor mRNAs with Different Histological Localization in the Gastrointestinal Tract

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Abstract. Two mouse urokinase-type plasminogen activator receptor (muPAR) cDNAs were isolated: muPAR1 is homologous to the human urokinase-type plasminogen activator receptor while muPAR2 codes for a 199 residue protein sharing the first 133 residues with muPAR1. Mouse genomic DNA sequencing indicates that the two different mRNAs arise by alternative splicing. In situ hybridization showed differential expression of the two mRNAs in mouse gastric mucosa. muPAR1 mRNA is located in luminal epithelial cells situated close to urokinase-type plasminogen activator–producing connective tissue cells of the lamina propria, pointing to plasmin generation controlled by the cooperation of different cells that may play a role in the release of gastric epithelial cells. muPAR2 mRNA is expressed in the basal epithelial cells, and the deduced protein sequence includes the receptor ligand binding domain, but omits the region involved in glycolipid-mediated membrane anchoring, suggesting that muPAR2 may code for a secreted uPA binding protein.

PLASMIN-mediated extracellular proteolysis generated by the urokinase-type plasminogen activator (uPA)[1] is involved in a variety of normal and pathological processes that require cell migration or tissue destruction, such as involution of the mammary (Ossowski et al., 1979; Larsson et al., 1984) and prostate (Andreasen et al., 1990*b*) glands, trophoblast invasion (Strickland et al., 1976; Sappino et al., 1989), spermatogenesis (Vihko et al., 1988), wound healing (Grøndahl-Hansen et al., 1988), and cancer invasion (Reich, 1978; Ossowski and Reich, 1983; Skriver et al., 1984; Danø et al., 1985; Bergman et al., 1986; Mignatti et al., 1986; Saksela and Rifkin, 1988; Grøndahl-Hansen et al., 1991). The regulation of the uPA pathway of plasminogen activation is quite complex. uPA is released as a virtually inactive single chain pro-enzyme, which by limited proteolysis is converted to two-chain active uPA. The uPA activity is regulated both temporally and spatially in time and space by two specific plasminogen activator inhibitors, PAI-1 and PAI-2 (Seifert and Gelehrter, 1978; Schleef and Loskutoff, 1988; Andreasen et al., 1990*a*), and by a specific cell surface receptor for uPA (Vassalli et al., 1985; Stoppelli et al., 1985).

The human uPA receptor (huPAR) has been purified and characterized, and a complete cDNA has been isolated. It is a cysteine-rich, highly glycosylated 55–60,000 M_r protein, synthesized as a 313 amino acid residue peptide preceded by a signal peptide (Nielsen et al., 1988; Estreicher et al., 1989; Behrendt et al., 1990; Roldan et al., 1990), which is anchored to the cell surface by a phosphatidylinositol glycolipid anchor (Ploug et al., 1991*b*). The mature huPAR consists of three repeats which appear to represent different domains, the NH_2-terminal 87 residues constituting the uPA binding domain (Behrendt et al., 1991). The human receptor is present on the surface of a variety of cultured cell lines of neoplastic and non-neoplastic origin (Blasi et al., 1987; Blasi, 1988; Nielsen et al., 1988). huPAR mRNA has been identified in colon cancer cells in situ at invasive foci, adjacent to uPA producing stromal cells (Grøndahl-Hansen et al., 1991; Pyke et al., 1991). huPAR binds human uPA with a high affinity that varies between cell types. Concomitant binding of pro-uPA to uPAR and of plasminogen to as yet unidentified binding sites strongly enhances plasmin generation on cell surfaces (Plow et al., 1986; Ellis et al., 1989, 1990; Stephens et al., 1989). In some cell types, receptor-bound pro-uPA is localized on cell–cell and focal cell–substratum contact sites (Pöllanen et al., 1988; Herbert and Baker, 1988).

We have previously studied murine uPA (Danø and Reich, 1978; Danø et al., 1980; Skriver et al., 1982) and the occurrence of this protein and its mRNA in situ under both normal (Larsson et al., 1984; Vihko et al., 1988; Andreasen et al., 1990*b*; Kristensen et al., 1991) and pathological conditions, including wound healing (Grøndahl-Hansen et al., 1988) and cancer invasion (Skriver et al., 1984; Kristensen et al., 1990). No structural information has been reported about the mouse uPA receptor (muPAR), in contrast to the increas-

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1. *Abbreviations used in this paper*: GPI, glycosyl-phosphatidyl-inositol; huPAR, human urokinase-type plasminogen activator receptor; muPAR, mouse urokinase-type plasminogen activator receptor; uPA, urokinase-type plasminogen activator; PCR, polymerase chain reaction.

ing amount of information available concerning huPAR. Characterization of muPAR is an important basis for performing functional studies of the plasminogen activation system in the mouse and to achieve this goal we have now isolated cDNA encoding the mouse receptor and surprisingly found two different types of muPAR mRNA, which are produced by alternative splicing and are differentially expressed in the mouse gastrointestinal tract as shown by in situ hybridization.

Materials and Methods

cDNA Cloning

Two fragments of the huPAR cDNA (Roldan et al., 1990) were used for screening: PstI fragment (268 bp) (bp 185–453) and BamHI fragment (585 bp) (bp 498–1083). DNA fragments were labeled with ^{32}P-dCTP by using a random priming kit (Boehringer Mannheim Biochemicals, Indianapolis, IN), and hybridization conditions were determined by Southern hybridization to human and mouse genomic DNA. 1.5×10^6 plaques from a mouse macrophage lambda gt11 library (Clontech, Palo Alto, CA) were screened on duplicate Genescreen filters (NEN Dupont, Boston, MA) at 42°C in 35% formamide, washed at increasing stringency until 47°C in 0.1 × SSC with 1% SDS, and 62 plaques hybridizing with the BamHI fragment on both duplicates were identified. After removal of bound probe by boiling, filters were rescreened with the PstI fragment under the same conditions and 82 positive plaques were found. A comparison showed that 22 plaques hybridized with both the BamHI and PstI fragment. Four lambda clones were purified by three rounds of plaque purification, and inserts were subcloned into a plasmid vector (pBluescriptKS(+))(Stratagene, La Jolla, CA) and analyzed by restriction mapping and dideoxynucleotide DNA sequencing with Sequenase (UBS, Cleveland, OH) with a combination of nested Exonuclease III deletions and synthetic oligonucleotide primers. Sequence homology searching was performed using the FastA/TFastA methods (Pearson and Lipman, 1988) as implemented in the GCG package (Devereux et al., 1984), using word sizes of six and one, respectively.

In Vitro Translation

Plasmid DNA containing the muPAR1 or muPAR2 cDNA was linearized with SspI, and sense RNA was prepared in transcription reactions containing: 2.5 μg DNA template, 40 mM Tris-HCl, pH 7.6, 6 mM $MgCl_2$, 10 mM NaCl, 2 mM spermidine, 10 mM DTT, 0.5 mM of each ribonucleotide, and 60 U of the appropriate polymerase (T3/T7) (Promega Biotech, Madison, WI). After incubation for 120 min at 37°C, 5 U of RQ1YDNase (Promega Biotech) was added and incubation continued at 37°C for 15 min. After phenol/chloroform extraction, the RNA was precipitated with ethanol and redissolved in diethylpyrocarbonate-treated water. One fifth of the generated RNA was used for in vitro translation using ^{35}S-labeled cystein (Promega Biotech). 1/50 of the translation reaction was analyzed by SDS-PAGE and fluorography.

PCR Amplifications

1 μg of mouse genomic DNA (Balb/c) (Clontech) was added to a reaction containing 25 pmol primer 1:5′-GAGCTGTGAGAGGGGCCGG, 25 pmol primer 2: 5′-CCACAGCCTCGGGTGTAGTCCT, 1 mM of each deoxynucleotide, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$ and 1 U Perfect Match (Stratagene). After initial denaturation for 5 min at 94°C, 2.5 U of AmpliTaq polymerase (Perkin Elmer Cetus, Norwalk, CT) was added and amplification for 40 cycles of 94°C (30 s), 55°C (30 s), and 72°C (2 min) was performed. 2 U of Klenow polymerase (Boehringer Mannheim Biochemicals) was added to 1/10 of the PCR reaction and incubated for 15 min at 37°C. After agarose gel electrophoresis, the DNA was purified using Geneclean (Bio101, La Jolla, CA) and cloned into the SmaI site of pBluescriptKS(+). The DNA sequence was determined using the dideoxy method and synthetic oligonucleotides.

RNA was isolated from mouse stomach mucosa (fundus or antrum part) according to the method of Chomczynski and Sacchi (1987). 5 μg of RNA was used for first strand cDNA synthesis in a reaction containing 25 pmol primer 2, 2.5 mM of each deoxynucleotide, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 10 U of RNasin (Promega Biotec), and 60 U of AMV reverse transcriptase (Promega Biotec). The reaction was incubated at 23°C (10 min), 42°C (60 min), and 94°C (5 min). The reaction was then diluted fivefold maintaining Tris, KCl, and $MgCl_2$ concentrations and after addition of primer 1 (25 pmol) and 2.5 U of AmpliTaq polymerase amplified for 40 cycles at 94°C (1 min), 55°C (1 min), and 72°C (2 min). The ~330 nucleotide DNA fragment was purified from the agarose gel and reamplified using the same conditions. 5 μl of this second reaction was used for asymmetrical amplification using 100 pmol of one primer and 0.5 pmol of the other, or reverse. The products of the asymmetrical reaction were purified by Geneclean and each sequenced using the dideoxynucleotide method using the primer present in low amount in the preceding asymmetrical amplification.

In Situ Hybridization

RNA probes were generated from three subclones of the cDNA in pBluescriptKS(+). The NH_2-terminal XhoI fragment was subcloned generating a plasmid containing bases 1–366. By PCR amplification with primer 1 and 2 (as described above) a second fragment including bases 512–698 of muPAR2 was isolated and subcloned into the SmaI site of pBluescriptKS(+). The COOH-terminal probe common to both muPAR1 and muPAR2 was generated by PCR amplification between nucleotide 701–1186 of muPAR1, using 18-mer oligonucleotides with added synthetic BamHI or HindIII sites and subcloned into pBluescriptKS(+) digested with these enzymes. The DNA sequence and orientation of the inserts in the three clones were confirmed by DNA sequencing. RNA probes for detection of uPA mRNA was generated from the plasmid pMUPA07 (Kristensen et al., 1991). ^{35}S labeled antisense and sense RNA was prepared from linearized templates and used for in situ hybridization of cryostat sections as described (Kristensen et al., 1991).

Results

Isolation and Characterization of muPAR cDNA

Two non-overlapping fragments from the coding region of human uPA receptor cDNA were used sequentially to screen a mouse macrophage lambda gt11 cDNA library under non-stringent conditions. 22 plaques hybridized with both fragments and 4 were plaque purified. DNA sequencing and restriction enzyme analysis demonstrated that one clone (designated muPAR1) resembles the known huPAR cDNA, while the other three (muPAR2) were similar to muPAR1 except for two regions: an insertion of 218 bp at nucleotide number 480 and start of the polyadenylated region at an earlier point than muPAR1 (Fig. 1). Two of the muPAR2 cDNAs were completely identical, while one had an additional adenosine in the polyA(+) tail.

The muPAR1 cDNA sequence is homologous to that of huPAR (Roldan et al., 1990), diverging however significantly in the 3′ untranslated region (Fig. 1). The muPAR1 cDNA is probably incomplete as it contains 453 nucleotides after the translation termination codon TGA, but no poly-A tract. It codes for a 304 amino acid protein, with a putative signal peptide of 22 residues. In comparison with huPAR, muPAR1 has two additional amino acids at positions 83 and 258, but lacks three amino acids following residue 134, seven amino acids following residue 273, and one after residue 299. As shown in Fig. 1, all cysteine residues are conserved; overall muPAR1 and huPAR have 62% amino acid identity and 70% homology when conservative substitutions are included. The COOH-terminal sequence of the muPAR1 protein is slightly more hydrophobic than huPAR but probably still insufficient for transmembrane attachment of this receptor. It is thus likely that muPAR1 is attached to the membrane by the phosphatidyl inositol mechanism as demonstrated for huPAR (Ploug et al., 1991*b*).

In the muPAR2 cDNA 218 nucleotides are inserted at position 480. Following this insertion the DNA sequence again becomes identical to that of muPAR1, and the identity con-

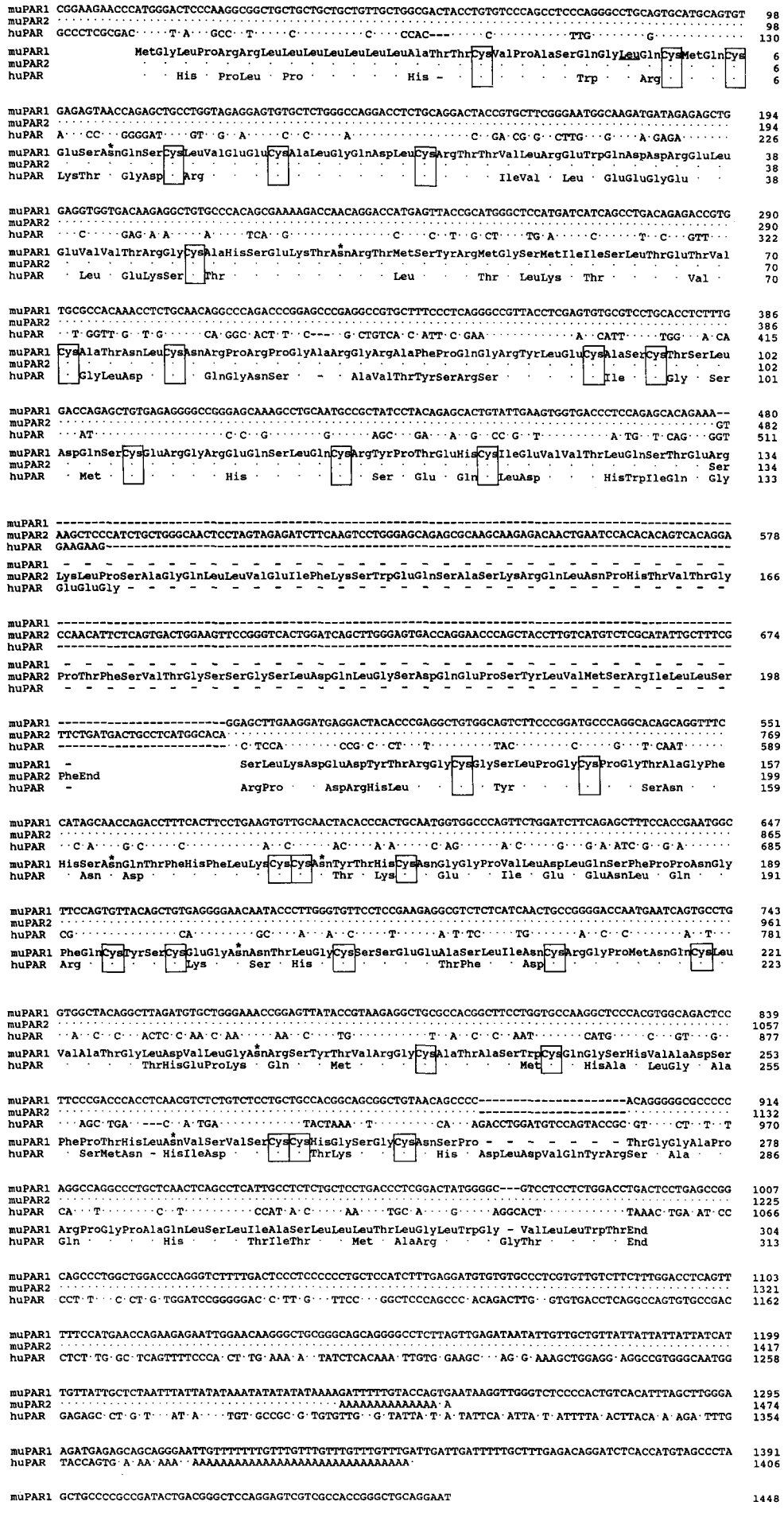

Figure 1. Sequence of the two muPAR cDNAs isolated and the amino acid sequence of the corresponding proteins as predicted from the cDNA sequence data. The data are aligned with the uPAR DNA and protein sequences (Roldan et al., 1990). The NH_2-terminal of the mature protein is underlined, potential N-linked glycosylation sites of muPAR1 are indicated by asterisks (*), and the conserved cysteine residues are boxed. The sequence data are available from EMBL/GenBank/DDJB under accession numbers 62700 (muPAR1) and 62701 (muPAR2).

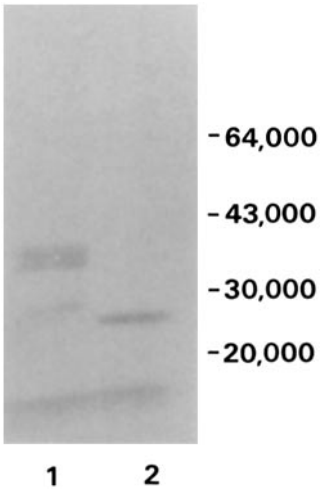

Figure 2. SDS-PAGE of in vitro translation products generated using rabbit reticulocyte lysate, ^{35}S-Cystein, and synthetic RNA generated from the two mouse uPA receptor cDNAs. (Lane *1*) muPAR1 mRNA; (lane *2*) muPAR2 mRNA. Size of molecular weight markers is indicated.

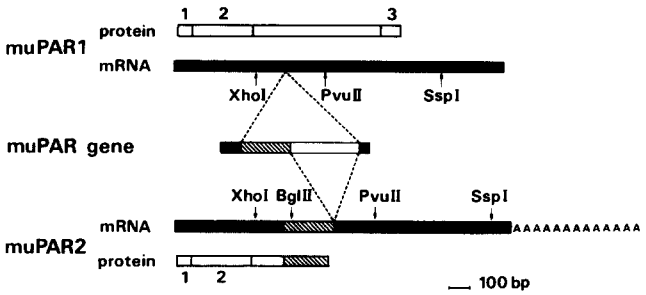

Figure 4. Schematic representation of the relationship between the partial genomic clone, the isolated muPAR1 and muPAR2 cDNAs, and the predicted two different uPAR binding proteins. (*1*) Predicted signal sequence. (*2*) Domain homologous to the region of huPAR responsible for uPA binding. (*3*) Region homologous to the part of huPAR removed during posttranslational attachment of glycolipid anchor. The hatched bar represents the DNA/protein sequence unique to muPAR2 and the white bar in the muPAR gene represents the intron sequence spliced out in both muPAR1 and muPAR2.

tinues until nucleotide 1240 of muPAR1. At this point, a short (14 nucleotide) stretch of A's is present, which makes a 20 nucleotide long poly A-tail, terminating the muPAR2 cDNA. The insertion contains two stop codons in tandem (nucleotide 678–683) and the muPAR2 cDNA therefore only codes for a total of 199 amino acids. The protein shares the first 133 amino acid residues with muPAR1, followed by 66 additional residues which are unique for muPAR2 (Fig. 1). The 66 amino acid stretch encoded by this insertion is not particularly hydrophobic. The absence of cysteines from this sequence is noteworthy, as this leaves an uneven number of cysteine residues in the protein. The inserted DNA and protein sequences do not shown significant homology to known nucleic acid or protein sequences in the GenBank/EMBL database.

In Vitro Translation

The insertion of 218 bp found in the muPAR2 cDNA leads to the premature termination of the receptor protein. Complete cDNA sequence of this insert region was determined on both strands in all three cDNA clones and found to be identical; the presence of the stop codons was further verified by in vitro translation of synthetic mRNA prepared from the muPAR1 and muPAR2 cDNA. Indeed, it was demonstrated that while muPAR1 codes for translation products with a predominant molecular weight of ~35,000, the muPAR2 cDNA only codes for a protein with a molecular weight of ~24,000 (Fig. 2), confirming that the insertion in muPAR2 leads to an earlier termination of protein translation. No microsomes were added to the translation reaction so, presumably, the doublet observed with muPAR1 RNA is the result of incomplete translation.

The Two Forms of muPAR mRNA Arise by Alternative Splicing

A DNA clone containing mouse genomic DNA was isolated by PCR amplification with two primers located on either side of the point of insertion in muPAR2 (see Materials and Methods). The isolated DNA fragment was blunt-ended with Klenow fragment, subcloned into pBluescriptKS(+), and sequenced (Fig. 3). Three base pair changes were found in the genomic clone. These substitutions would change Gly(177) to Arg(177) and Arg(194) to Pro(194). However, since the sequence of the muPAR2 cDNA specific region was determined in three cDNA clones where at least two are independent clones, it is most likely that these differences are caused by errors of the Taq polymerase early in the amplification procedure. The DNA sequence (Fig. 3) shows that

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gene    TGAGAGGGGCCGGGAGCAAAGCCTGCAATGCCGCTATCCTACAGAGCACTGTATTGAAGTGGTGACCCTCCAGAGCACAGAAAGTAAGCT  90
muPAR2  ....................................................................................... 487
                                                                                        *

gene    CCCATCTGCTGGGCAACTCCTAGTAGAGATCTTCAAGTCCTGGGAGCAGAGCGCAAGCAAGAGACAACTGAATCCACACACAGTCACGGG 180
muPAR2  .....................................................................................A.. 577

gene    ACCAACATTCTCAGTGACTGGAAGTTCCCGGTCACTGGATCAGCTTGGGAGTGACCAGGAACCCAGCTACCTTGTCATGTCTCCCATATT 270
muPAR2  ..........................G.....................................................G..... 667

gene    GCTTTCGTTCTGATGACTGCCTCATGGCACAGTATGGCCACCATAATTCCAGACTTGGTGTCCACATTCCAGAGAGAAGAAAAGGGGAAA 360
muPAR2  ..............................------------------------------------------------------- 698

gene    GAAGGGCCCCAAAATTCCTTTGTTTTGTAAGGGTTTTCTTTCTAGAAATCCCAGAAACGCATAAGGATCTATTTATCGTTCGGGCCTCCT 450
muPAR2  ---------------------------------------------------------------------------------------

gene    TTATCTATAACAAAGGGGAAAAGGAACTCGAAGCCCTTTGGCTAAGGATGAAGTTACCCTAAGTAGCAAGGGCCTCTGATAAAAGGGGTG 540
muPAR2  ---------------------------------------------------------------------------------------

gene    TGCGCAAGGGGGCAAATCAACACCCAGCCCCTTACCAGGATCTGCCCTCTGCCTTCTGCAGGGAGCTTGAAGGATGAGGACTACACCCGA 630
muPAR2  ------------------------------------------------------------.......................... 727
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Figure 3. DNA sequence of a partial mouse genomic DNA clone isolated by PCR amplification. Base pairs differing in the gene from muPAR2 sequence are shown. The splicing point responsible for the difference between muPAR1 and muPAR2 is indicated by an asterisk (*) and the sequence not found in muPAR1 and muPAR2 is indicated by dashes. The sequence found in the 3′ end of the utilized primers is underlined. This sequence is available from EMBL/GenBank/DDBJ under accession number 62702.

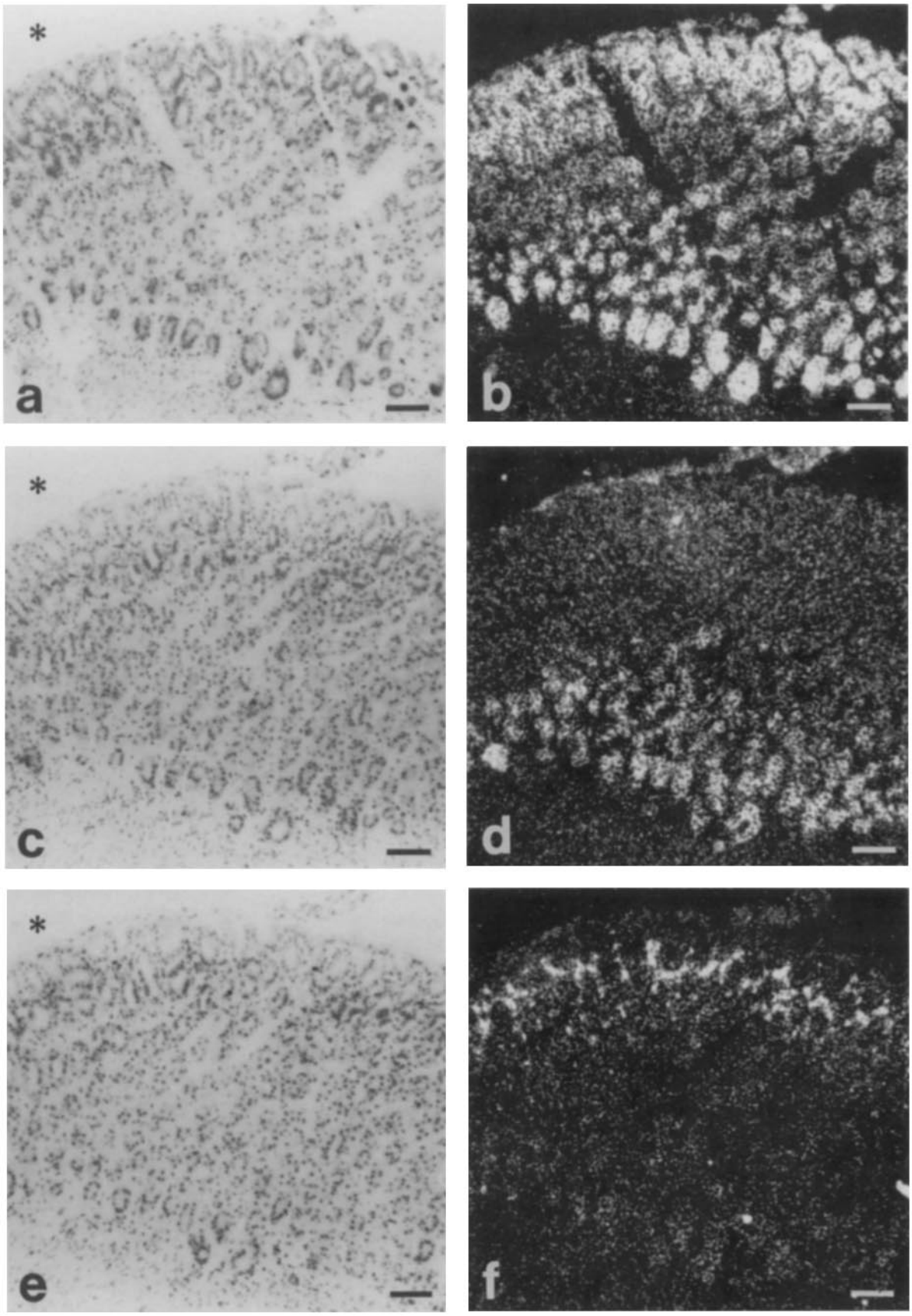

Figure 5. In situ hybridization analysis of adjacent cryostat sections of mouse ventricular mucosa using ^{35}S-labeled antisense RNA from an NH_2-terminal subclone common to both mouse uPA receptor mRNAs (muPAR1 and muPAR2) (*a* and *b*), antisense RNA specific for muPAR2 (*c* and *d*) or mouse uPA antisense RNA (e and *f*). After hybridization sections were covered with emulsion and exposed for 7 d. Sections are viewed by either transmitted light (*a*, *c*, and *e*) or using dark-field illumination (*b*, *d*, and *f*). Asterisks indicate gut lumen. Bar, 100 μm.

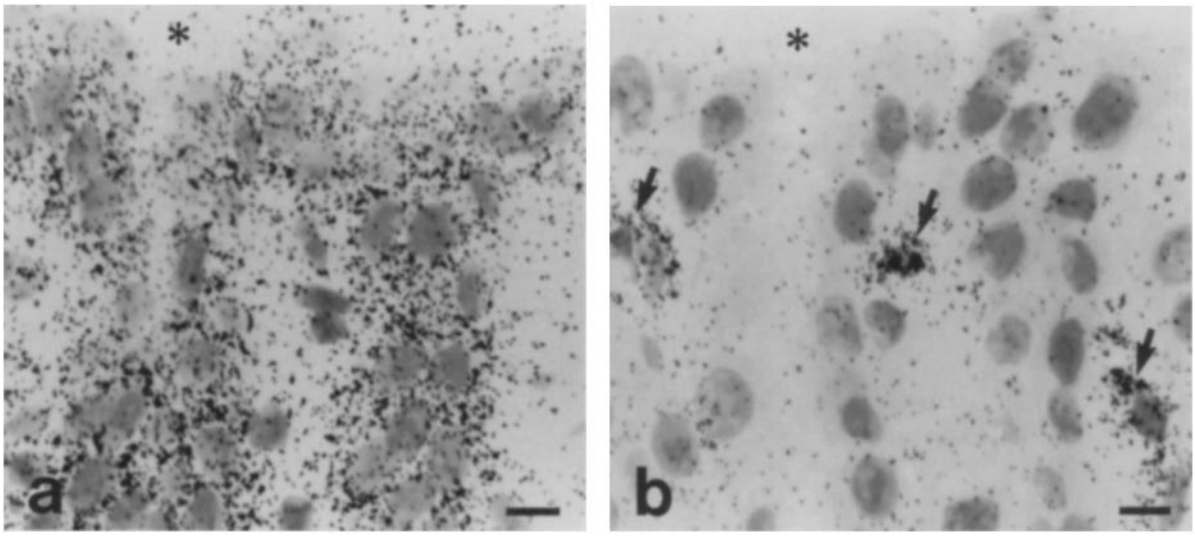

Figure 6. High power magnification of two adjacent sections from the luminal part of the mouse ventricular mucosa hybridized with ^{35}S-labeled antisense RNA from an NH_2-terminal subclone common to both mouse uPA receptor mRNAs (*muPAR1* and *muPAR2*) (*a*) or mouse uPA mRNA (*b*). Note that while signal for the uPA receptor mRNA is found over the entire luminal epithelium, the signal for the corresponding ligand is found over distinct fibroblast-like cells just below in the lamina propria (*arrows* in *b*). Asterisks indicate gut lumen. Bars, 10 μm.

following the employed primer the genomic clone starts with 70 nucleotides that are common to both muPAR1 and muPAR2, continues with the 218 nucleotides unique to muPAR2 followed by 300 nucleotides, and finally returns again to sequence common to both muPAR1 and muPAR2 (now 3′ untranslated region of the latter). We therefore conclude that muPAR1 and muPAR2 arise from a common precursor mRNA by alternative splicing. The same intron in fact can be spliced with two different donor sites generating two different mRNA species. The overall relation between the muPAR gene, muPAR1 and muPAR2 cDNA and muPAR1 and muPAR2 proteins is summarized in Fig. 4.

The Two muPAR mRNA Forms Are Differentially Expressed in the Gastrointestinal Tract

By restriction enzyme digestion or PCR amplification three subclones were prepared for generation of RNA probes for in situ hybridization (see Materials and Methods). Antisense RNA from two probes (designated NH_2-terminal and COOH-terminal probe) will detect both muPAR1 and muPAR2 mRNA, while the third (designated muPAR2 probe) will recognize only muPAR2 mRNA, as it contains only the DNA sequence unique for this clone. The authenticity of the generated constructions was confirmed by DNA sequencing of the subclones. The corresponding antisense and sense ^{35}S-labeled RNA probes were generated and used to analyze the distribution of the two types of mRNA in the gastrointestinal tract of the mouse. In situ hybridization with antisense RNA from the NH_2-terminal and COOH-terminal subclones demonstrated signal over the epithelium of both the luminal and basal parts of the gastric mucosa in the fundus (Fig. 5, *a* and *b*), as well as in the antrum parts (not shown). However, when the muPAR2-specific probe was employed, the signal was only observed over the basal epithelial cells (Fig. 5, *c* and *d*). The luminal epithelial cells, therefore, most likely contain only the muPAR1 mRNA, while the basal epithelial cells may contain either a mixture of muPAR1 and muPAR2 or only muPAR2 mRNA. Interestingly, mouse uPA mRNA is found in distinct fibroblast-like cells situated luminally in the lamina propria (Fig. 5, *e* and *f*), where also the mouse uPA protein previously was found (Larsson et al., 1984). Examination of adjacent sections at a higher magnification demonstrates that the epithelial cells that contain signal for the NH_2-terminal receptor probe, are situated in close proximity to the fibroblast-like cells containing the signal for mouse uPA (Fig. 6). Control experiments performed by hybridization of adjacent sections with the same amount of the corresponding sense RNA probes gave no signal (results not shown). Hybridization to cryostat sections of other parts of the gastrointestinal tract (antrum, duodenum, and jejunum) showed a similar pattern: the NH_2-terminal probe giving signal over both luminal and basal parts of the epithelium, while the muPAR2-specific probe only shows signal over the basal parts of the epithelium (results not shown).

To further demonstrate the presence of muPAR mRNA, RNA was isolated from mouse gastric mucosa (antrum and fundus) and used as template for first strand cDNA synthesis. PCR amplification using primers flanking 391–515 bp in muPAR1 and 391–733 bp in muPAR2 showed the presence of two bands of ~340 and 120 bp as observed after agarose gel electrophoresis (data not shown). The ~340-bp band was isolated from the agarose gel and used as template for asymmetrical PCR amplification. Dideoxy nucleotide sequencing of the resulting two single-stranded DNA preparations revealed sequence identical to that of the muPAR2 cDNA (219 bases downstream from basepair number 432 and 209 bases upstream from basepair number 639 of muPAR2), demonstrating the presence of the muPAR2 mRNA in the mouse fundus RNA preparation.

Discussion

The existence of a uPA receptor in the mouse has previously been indicated by binding studies with mouse cell lines as

well as mouse spermatozoa (Huarte et al., 1987; Estreicher et al., 1989). The strong homology between muPAR1 and huPAR allows us to conclude that the mouse (type 1) uPA receptor has characteristics similar to the human. A prominent feature of the huPAR is its carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidyl-inositol (GPI) (Ploug et al., 1991*b*). Characteristic for GPI-anchored proteins is the presence of a moderately hydrophobic region of at least 17 amino acid residues close to the COOH-terminal end of the nascent protein (Ferguson and Williams, 1988). In muPAR1 a hydrophobic region starts at residue 280 of muPAR1 and goes through to the COOH-terminal (residue 304) with no interrupting charged residues. However, it must be noted that seven residues are deleted from muPAR1 when compared to huPAR (residues 275–281 in huPAR) immediately NH_2-terminal to the region predicted to be the site of GPI anchoring in huPAR (residues 282–284) (Ploug et al., 1991*b*). Amino acids with small side chains (Gly, Asn, Cys, Ser, or Asp) are preferred at the GPI attachment site as well as at the adjacent two COOH-terminal residues (Micanovic et al., 1988; Berger et al., 1988). On this basis we propose that the putative GPI-anchor in muPAR1 is attached at Gly (275) or close to this residue.

The huPAR is a highly glycosylated protein, the deglycosylated mature polypeptide chain comprising only ~35 kD compared with the apparent molecular mass (55–60 kD) of the mature glycoprotein (Behrendt et al., 1991). The five putative N-glycosylation sites in the human receptor (Roldan et al., 1990) are all present with identical localization in muPAR1, which in addition has two other potential N-glycosylation sites (Fig. 1). In common with the human receptor is also a very high cysteine content in muPAR1 (~9% in the mature protein in both cases). This may reflect a high number of intra-chain disulphide bridges defining compact domain structures. Together with the high degree of glycosylation, this feature may render the receptors very resistant to proteolysis, and thus well suited to function in a proteolytic environment (Ploug et al., 1991*a*).

The huPAR consists of three repeats with low level internal homology particularly in the number and location of the cysteine residues (Behrendt et al., 1991). These repeats are also found in the muPAR1 protein and by analogy it is likely that the uPA binding domain is also located in the NH_2-terminal repeat, i.e., within residues 1–87. The consensus sequence of the three repeats in huPAR has the same cysteine spacing pattern as three other GPI-anchored proteins (the Ly6a and Ly6b lymphocyte activating antigens (LeClair et al., 1986; Palfree et al., 1987; Palfree et al., 1988; Reiser et al., 1988) and the squid glycoprotein Sgp-2 (Williams et al., 1988). All three repeats in muPAR1 share these homologies, strengthening the assumption that the homologies are of structural significance and that they reflect the existence of a novel domain characteristic of a protein family, to which huPAR and muPAR1 belong. The human and mouse u-PA receptors are quite species specific with respect to ligand binding (Appella et al., 1987; Huarte et al., 1988; Estreicher et al., 1989). A number of differences are found in the 1–87 region, that may account for this species specificity (Fig. 1).

The presence of muPAR1 mRNA in luminal epithelial cells at the neck of the glands in the gastric mucosa probably reflects the presence of type 1 mouse uPA receptor. Mouse uPA mRNA and protein is present in adjacent fibroblast-like connective tissue cells (Fig. 6) (Larsson et al., 1984; Kristensen et al., 1991), and it is likely that uPA produced by these cells is secreted and subsequently bound to the uPA receptor on the epithelial cells; the receptor-bound uPA may then provide proteolytic activity at the cell surface during the continuous shedding of the latter cells, in keeping with our previous proposal (Larsson et al., 1984; Kristensen et al., 1991).

Recently, a similar cellular cooperation in the control of plasmin generation has been found in human colon cancer, where uPA is produced by one cell type (fibroblast-like cells in the tumor stroma), while the uPA receptor is produced by cancer cells at invasive foci (Grøndahl-Hansen et al., 1991; Pyke et al., 1991). Likewise, a paracrine mechanism involving secretion of uPA from one cell type and subsequent binding to the receptor on another cell type has been proposed for the uPA produced by epithelial cells in the vas deferens of the mouse (Larsson et al., 1984; Huarte et al., 1987) and bound by spermatozoa, presumably through the uPA receptor. Together with the strong enhancement of cell surface plasmin generation observed after receptor binding of pro-uPA in vitro (Ellis et al., 1989, 1990; Stephens et al., 1989), and the fact that cell-surface bound plasmin is resistant to its physiological inhibitor alpha-2-anti plasmin (Plow et al., 1986; V. Ellis, personal communication), these findings suggest that the binding of uPA produced by one cell type to uPA receptors on another cell type may play a role for physiological generation of cell surface localized proteolytic activity.

Genomic DNA sequencing shows that muPAR1 and muPAR2 mRNA arise by alternative splicing of pre-mRNA transcribed from the same gene. Excision of the same intron occurs at different positions in the two cases, the upstream donor splicing point being different and the downstream acceptor point being identical, as also seen in other cases of alternative splicing (Breitbart et al., 1987).

The additional nucleotide sequence in the type 2 muPAR mRNA contains two stop codons in tandem giving rise to a protein containing the supposed binding domain for uPA, but no hydrophobic region, thus, lacking a membrane-spanning domain or a GPI-anchoring signal. muPAR2 may therefore be a secreted, water-soluble, uPA-binding protein. Soluble forms of membrane bound proteins have previously been reported and in some cases it has been shown that they arise by alternative splicing of pre-mRNA: examples are the neural adhesion molecule NCAM (Gower et al., 1988), the growth hormone receptor (Baumbach et al., 1989), the EGF receptor (Petch et al., 1990), and the transferrin receptor (Shih et al., 1990).

The mRNA coding for muPAR2 is expressed in intact mouse tissue: the basal epithelial cells in all analyzed parts of the gastrointestinal tract show signal for muPAR2 as demonstrated by in situ hybridization using an antisense RNA probe specific for muPAR2. The corresponding sense-RNA probe showed no signal when an equal amount of probe was added to adjacent sections, giving only a background hybridization pattern similar to that found with uPA sense and antisense probes outside the fibroblast-like cells of the gastric mucosa (Kristensen et al., 1991). Northern blot experiments with RNA isolated from the gastric mucosa did not give signal, presumably due to insufficient amounts of RNA. To substantiate the presence of muPAR2 mRNA in this tissue we therefore used the RNA for cDNA synthesis and PCR

amplification. The DNA sequence of the largest resulting fragment was determined and found to be identical to the muPAR2 sequence. The primers employed were identical to those used for isolation of the genomic clone, thus, spanning the intron and assuring that we were not amplifying genomic DNA. The results in this study clearly demonstrate that there are two types of muPAR mRNA encoded by the same gene and generated by alternative splicing. The protein sequences predicted from the two mRNAs are different and both mRNAs are expressed in the normal mouse with a different distribution. That both mRNAs are translated in vivo appears likely, but remains to be demonstrated. The fact that mouse uPA is not readily cross-linked to its receptor (Estreicher et al., 1989; H. Solberg and G. Høyer-Hansen, personal communication), as is the case for huPA-huPAR, complicates the demonstration of the muPAR2 protein. However, generation of muPAR1 and muPAR2 specific antibodies on the basis of the data presented here, will hopefully clarify this question.

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