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(19) (CA) **CANADIAN PATENT** (12)

(54) DNA Clones of Human Placental Plasminogen Activator Inhibitor

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DNA CLONES OF HUMAN
PLACENTAL PLASMINOGEN ACTIVATOR
INHIBITOR

Abstract of the Disclosure

5 cDNA clones having a base sequence for
human placental plasminogen activator inhibitor
(PAI-2) have been developed and characterized and the
amino acid sequeunce of the PAI-2 has been determined.
The PAI-2 protein has then been expressed in
10 prokaryotic and eukaryotic cells.

DNA CLONES OF HUMAN
PLACENTAL PLASMINOGEN ACTIVATOR INHIBITOR

Background of the Invention

5 This invention relates to a plasminogen activator inhibitor and, more particularly, to cDNA clones representing essentially a full size plasminogen activator inhibitor of the placental type and to expression of the recombinant protein in prokaryotic and eukaryotic hosts.

10 The plasminogen activators are a class of serine proteases that convert plasminogen to the fibrinolytically active enzyme plasmin (fibrinolysin). Upon being thus activated, the plasmin can attack the coagulation proteins of the
15 fibrin clot (thrombus) and thereby disintegrate the clot. Inhibitors normally present in the blood with plasminogen generally retard this reaction.

Human plasma contains two plasminogen activators that are immunologically distinct, namely
20 tissue plasminogen activator (t-PA) and urokinase (u-PA). t-PA has been demonstrated to have higher affinity for fibrin than u-PA and, therefore, is a preferable agent for degradation of the fibrin clot. The source of the plasma t-PA has been presumed to be
25 the vascular endothelium.

Plasminogen activator inhibitors (PAI) have been obtained from various sources. They are now classified in at least three immunologically different groups: protease nexin-I, the endothelial
30 cell type plasminogen activator inhibitor (PAI-1), and the placental type plasminogen activator inhibitor (PAI-2).

Protease nexin-I was isolated from human fibroblasts and has an apparent M_r 43 kilodaltons
35 (kDa). Scott et al., J. Biol. Chem. 260 (11),

7029-7034 (1985). It is distinguished by its acid lability, its ability to inhibit both plasminogen activators and plasmin, its relatively high pI (7.5-7.8) and by the stimulatory effect of heparin on its activity.

Endothelial cell-type PAI-1 is a glycoprotein of M_r 50-54 kDa that rapidly inactivates both t-PA and μ -PA. It is synthesized by endothelial cells and certain hepatoma and fibrosarcoma lines. It is found in platelets and is believed to constitute the major PAI of normal human plasma. See Pannekoek et al., The EMBO Journal 5 (10), 2539-2544 (1986); Andreason et al., FEBS Lett. 209 (2), 213-218 (1986); Ginsburg et al., J. Clin. Invest. 98, 1673-1680 (1986); and Wun and Kretzmer, FEBS Lett. 210, 11-16 (1987).

Placenta-type PAI-2 is a distinct protein of M_r 47,000. Kawano et al., Nature 217, 253-254 (1968); Astedt et al., Thromb. Haemostasis 53, 122-125 (1985); and Wun and Reich, J. Biol. Chem. 262, 3646-3653 (1987). It appears to be immunologically and biochemically identical to a similar activity of human monocytes [Chapman et al., Cell 28, 653-662 (1982), and Kopitar et al., Thromb. Haemostasis 54, 750-755 (1985)], and monocytic cell lines [Vassalli et al., J. Exp. Med. 159, 1653-1668 (1984), and Kruithof et al., J. Biol. Chem. 261, 11207-11213 (1986)].

Further background information on the plasminogen activator inhibitors can be had by reference to the recent review article by Sprengers and Kluft, Blood 69(2), 381-387 (1987).

Recent advances in biochemistry and in recombinant DNA technology have made it possible to synthesize specific proteins, for example, enzymes, under controlled conditions independent of the organism from which they are normally isolated.

These biochemical synthetic methods employ enzymes and subcellular components of the protein synthesizing systems of living cells, either in vitro in cell-free systems, or in vivo in microorganisms. In either case, the principal element is provision of a deoxyribonucleic acid (DNA) of specific sequence which contains the information required to specify the desired amino acid sequence. Such a specific DNA sequence is termed a gene. The coding relationships whereby a deoxyribonucleotide sequence is used to specify the amino acid sequence of a protein is well-known and operates according to a fundamental set of principles. See, for example, Watson, Molecular Biology of the Gene, 3d ed., Benjamin-Cummings, Menlo Park, Calif., 1976.

A cloned gene may be used to specify the amino acid sequence of proteins synthesized by in vitro systems. RNA-directed protein synthesizing systems are well-established in the art. Double-stranded DNA can be induced to generate messenger RNA (mRNA) in vitro with subsequent high fidelity translation of the RNA sequence into protein.

It is now possible to isolate specific genes or portions thereof from higher organisms, such as man and animals, and to transfer the genes or fragments to microorganisms such as bacteria (e.g., E. coli) or yeasts (e.g., S. cerevisiae). The transferred gene is replicated and propagated as the transformed microorganism replicates. Consequently, the transformed microorganism is endowed with the capacity to make the desired protein or gene which it encodes, for example, an enzyme, and then passes on this capability to its progeny. See, for example, Cohen and Boyer, U.S. Pats. 4,237,224 and 4,468,464. Likewise, mammalian cells (e.g., mouse, bovine, and Chinese hamster ovary) can be used for the expression of mammalian protein by conventional recombinant DNA

methods. See, for example, Axel et al., Science 209, 1414-1424 (1980) and U.S. Patent 4,399,216.

To illustrate, a bacterial plasmid, for example, pSC101 or pBR322 and derivatives thereof, can be used as a cloning vehicle to introduce a foreign or exogenous gene into the host bacteria. An illustrative host bacteria can be, for example, Escherichia coli K-12_x 1776, which is available from the American Type Culture Collection, Rockville, Maryland under accession number ATCC 31244. The plasmid can be cleaved with a restriction endonuclease or other DNA cleaving enzyme, for example EcoR I, to form a linear DNA fragment having an intact replicon and cohesive termini. A second DNA fragment having the desired exogenous or foreign gene and a given phenotypical property and complementary ligatable termini can be obtained from a foreign cell or chemically synthesized. This second DNA fragment is spliced with the first DNA fragment with a DNA ligase or other DNA ligating agent, for example T₄DNA Ligase, to form a completely closed and recircularized plasmid. The insertion of the second DNA fragment into the EcoR I site of the illustrative plasmid brings the expression of the genetic information under the control elements of the plasmid. The resulting recombinant plasmid is then used for transformation of the bacterial cell and allowed to replicate by growing the bacteria in a suitable culture medium. The desired transformants are then isolated by phenotypical trait differentiation, for example, by resistance to particular growth-inhibiting materials such as antibiotics or by various morphological property differences.

Brief Description of the Invention

In accordance with the present invention, the complete coding sequence of cDNA clones representing essentially a full size plasminogen activator inhibitor of the placental type (PAI-2) has been developed. Active recombinant PAI-2 protein was expressed in prokaryotic and eukaryotic hosts by operably inserting the PAI-2 coding sequence in replicable expression vectors.

Initially, two nearly full-length cDNAs for the PAI-2 were isolated from a human placenta λ gt11 cDNA library. One positive, λ PAI-75.1, expressed a protein that could adsorb and purify anti-PAI antibodies. The expressed protein inhibited the activity of human urokinase in a fibrin autography assay, and formed a 79-kDa (reduced) covalent complex with ^{125}I -urokinase that could be immunoprecipitated with anti-PAI. The largest clone, λ PAI-75.15, contains a 1909 base pair (bp) cDNA insert with a 5'-noncoding region of 55 bp, an open reading frame of 1245 bp, a stop codon, a 3'-noncoding region of 581 bp, and a poly(A) tail of 25 bp.

The cDNA sequence encodes a 46.6 kDa protein of 415 amino acids. The translated protein sequence is related to members of the serpin gene family, particularly ovalbumin and the chicken gene Y protein. Like these avian proteins, the PAI-2 appears to lack a cleavable NH_2 -terminal signal peptide. Residues 347-376 of the PAI-2 exactly match the 30 residues of the sequence reported for a PAI purified from the human monocytic cell line U-937 by Kruithof et al., supra.

There are three potential glycosylation sites in the PAI-2 protein with the sequence Asn-X-Ser/Thr, wherein X can be any of the common 20

amino acids. These sites are at amino acid positions Asn 75, Asn 115, and Asn 339.

5 The original source of the protein material for developing the PAI-2 cDNA was human placental tissue. Such tissue is widely available after delivery by conventional surgical procedures. cDNA libraries in the expression vector λ gt11, were constructed from human placenta RNA and screened for positive clones. The λ gt11 (lac5 nin5 cl857 S100) used herein is a
10 well-known and commonly available lambda phage expression vector. It's construction and restriction endonuclease map is described by Young and Davis, Proc. Natl. Acad. Sci. USA 80, 1194-1198 (1983).

15 The active recombinant PAI-2 protein was expressed in E. coli cells to illustrate a prokaryotic host and in mouse C-127 cells to illustrate a eukaryotic host. The PAI-2 coding sequence illustrated by λ PAI-75.1 was inserted into conventional expression vectors (plasmids) illustrated
20 by the trp-lac promoter vector, pKK 223-3, for production in E. coli and the bovine papilloma virus (BPV) vector for production in mouse cells. Many clones of these recombinant cells expressed relatively high levels of the PAI-2. The PAI-2 protein expressed
25 in mouse cells is believed to be glycosylated whereas the corresponding protein produced in E. coli is believed to be non-glycosylated. This is in accordance with the understanding that proteins anchored on, or secreted by eukaryotic cells are
30 generally glycosylated whereas most prokaryotic cells do not produce glycoproteins. In both cases, the PAI-2 protein was essentially free of other proteins or peptides of human origin.

Detailed Description of the Invention

While the specification concludes with claims particularly pointing out and distinctly claiming the subject matter regarded as forming the present invention, it is believed that the invention will be better understood from the following detailed description of preferred embodiments of the invention in conjunction with the appended drawings, in which briefly:

FIG. 1 shows urokinase inhibitory activities in λ gt11 fusion proteins in a fibrin-agar spot assay. Phage lysates were preincubated with anti-PAI antibody (+anti-PAI) or with preimmune serum (-anti-PAI) and then tested for PAI activity. Urokinase activity is indicated by the appearance of a clear lytic zone, and PAI activity is shown by abolishment of the lytic zone. Lysates tested were from λ PAI-75.1 (λ 75.1), λ PAT-75.2 (λ 75.2), λ PAI-T (λ T), λ PAI-R (λ R), λ PAI-89.2 (λ 89.2), λ PAI-89.3 (λ 89.3), λ gt11 without insert (λ gt11), and a bovine serum albumin control (BSA).

FIG. 2 shows the SDS-PAGE analysis of the complex formation between ^{125} I-urokinase and the PA inhibitors in the phage lysates. Phage lysates containing fusion proteins from six positive clones were incubated with ^{125} I-urokinase, immunoprecipitated by anti-placenta PAI antibodies, and electrophoresed on a 7.5% polyacrylamide gel, as described herein-after. Gel lanes were as follows: lane 1, PBB solution; lane 2, λ gt11 lysate; lane 3, λ PAI-T lysate; lane 4, λ PAI-R lysate; lane 5, λ PAI-75.1 lysate, lane 6, λ PAI-75.2 lysate; lane 7, λ PAI-89.2 lysate; lane 8, λ PAI-89.3 lysate. Molecular weights of complexes are indicated at the left. The band at the bottom of lane

5 represents the light chain of urokinase (M_r 20,000).

FIG. 3 shows a partial restriction map and sequencing strategy for the λ PAI-75.1 and λ PAI-75.15 inserts. The scale at the bottom indicates the nucleotide positions relative to the protein initiation codon. The thick bar represents the coding region for the 415 amino acids of the placental PAI. The restriction endonuclease sites shown were confirmed by digestion.

FIG. 4 shows the nucleotide sequence and translated amino acid sequences of the human placenta PAI cDNA. Nucleotides are numbered on the right-hand side. Nucleotide 1 was assigned to the A of the ATG that encodes the first methionine. Negative numbers refer to the 5'-noncoding region. Amino acids are numbered underneath the sequence. Potential N-linked glycosylation sites are marked by asterisks. The proposed reactive center is indicated by arrowheads. Potential polyadenylation signals are underlined. Sequence from nucleotides -52 to 1829 was derived from λ PAI-75.1 and was determined on both strands of the cDNA. The first three nucleotides (GTT) and the poly(A) tail were determined on one strand from the λ PAI-75.15 insert. The 1909 bp cDNA of FIG. 4 is split into Panels (A) and (B) of FIG. 4.

FIG. 5 shows the fibrin autography for screening PAI activity in λ PAI-75.1 transfected E. coli JM 105 cells. The λ PAI-75.1 transfected JM 105 cells were grown at 37° in LB medium containing 50 μ g/ml ampicillin to optical density (OD) = 0.7. After addition of 1 mM IPTG, the cells were further incubated for 2 hrs. Aliquots of 0.8 ml of cultures were centrifuged to pellet the cells, and the cells were resuspended in 80 μ l of phosphate buffered saline

(PBS). The suspensions were briefly sonicated to lyse the cells. Fifteen μ l of lysates were each mixed with 5 μ l of urokinase (5 CTA units/ml) and incubated for 30 min. at room temperature. Two μ l each of the mixtures were spotted on a fibrin-agar plate. The clones which expressed higher level of PAI completely inhibited the urokinase induced fibrin lysis while controls and those which expressed low levels of PAI showed fibrin lysis as evident by the clear lytic zone in 6 wells.

FIG. 6 is a diagrammatic representation which shows the structure of the bovine papilloma virus expression vector, pMON1123, which was used for the expression of PAI-2 protein in mouse C-127 cells in one embodiment of the invention. In this vector: BPV is the complete bovine papilloma virus genome, SV40L is the late poly(A) addition site of the SV40 virus, mMT is the mouse metallothionin I promoter, and pML2 is a derivative of the E. coli plasmid pBR322 with an animal viral insert.

Standard biochemical nomenclature is used herein in which the nucleotide bases are designated as adenine (A); thymine (T); guanine (G); and cytosine (C). Corresponding nucleotides are, for example, deoxyguanosine-5'-triphosphate (dGTP). Amino acids are shown either by three letter or one letter abbreviations as follows:

Abbreviated Designation			Amino Acid
5	A	Ala	Alanine
	C	Cys	Cysteine
	D	Asp	Aspartic acid
	E	Glu	Glutamic acid
	F	Phe	Phenylalanine
10	G	Gly	Glycine
	H	His	Histidine
	I	Ile	Isoleucine
	K	Lys	Lysine
	L	Leu	Leucine
15	M	Met	Methionine
	N	Asn	Asparagine
	P	Pro	Proline
	Q	Gln	Glutamine
	R	Arg	Arginine
20	S	Ser	Serine
	T	Thr	Threonine
	V	Val	Valine
	W	Trp	Tryptophan
	Y	Tyr	Tyrosine

Commonly available restriction endonuclease
used herein have the following restriction sequences
and (indicated by arrows) cleavage patterns:

5	SalI	↓ GTCGAC
		CAGCTG ↑
	PvuII	↓ CAGCTG
		GTCGAC ↑
	PstI	↓ CTGCAG
		GACGTC ↑
10	NsiI	↓ ATGCAT
		TACGTA ↑
	SspI	↓ AATATT
		TTATAA ↑
15	XbaI	↓ TCTAGA
		AGATCT ↑
	NdeI	↓ CATATG
		GTATAC ↑
	EcoRI	↓ GAATTC
		CTTAAG ↑
20	HindIII	↓ AAGCTT
		TTCGAA ↑
	BamHI	↓ GGATCC
		CCTAGG ↑

In order to illustrate specific preferred embodiments of the invention in greater detail, the following exemplary laboratory preparative work was carried out. Example 1 illustrates the development of the complete coding sequence of cDNA clones representing essentially the full size PAI-2 protein. Example 2 illustrates the active recombinant PAI-2 protein expression in E. coli and mouse C-127 cells although it will be appreciated that other bacterial and mammalian cell cultures and expression vectors can be used for similar such protein expression.

Example 1

Materials -- Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories or New England Biolabs. Calf intestine alkaline phosphatase, E. coli DNA polymerase I, and S1 nuclease were purchased from Boehringer Mannheim. T4 polynucleotide kinase and exonuclease III were obtained from New England Biolabs. Mung bean nuclease, T4 DNA polymerase and the Klenow fragment of E. coli DNA polymerase I were purchased from Bethesda Research Laboratories. [³²P]-labeled deoxyribonucleotides and deoxyadenosine 5'-[α-³⁵S]thiotriphosphate ([³⁵S]dATPαS) were from Amersham. Nitrocellulose filters were from Schleicher and Schuell. Goat anti-rabbit IgG horseradish peroxidase conjugate and IgG beads were obtained from Bio-Rad. The IgG beads were reconstituted in 5 ml of PBS solution before use. Deoxyribonucleotides and dideoxyribonucleotides for sequencing reactions, and oligo(dT)-cellulose were purchased from Pharmacia. Bovine thrombin was obtained from Parke-Davis. Plasminogen was isolated from human plasma using lysine-Sepharose[®] 4B chromatography [Deutsch and Mertz, Science 170, 1095-1096 (1970)]. Partially purified human urokinase (Winkinase[®]) was from Winthrop

Laboratories. Urokinase was further purified by benzamidine-Sepharose chromatography using Winkinase as starting material, as described by Wun et al., J. Biol. Chem. 257, 7262-7268 (1986). ^{125}I -urokinase

5 was prepared by a gentle chloramine T iodination method described by Wun and Capuano, Ibid 260, 5061-5066 (1985). Phorbol 12-myristate 13-acetate and lactalbumin hydrolysate were from Sigma. Trasylol[®] was purchased from American Diagnostica
10 Inc. RPMI and DME cell culture media were obtained from Gibco Laboratories. Oligonucleotides were synthesized on an Applied Biosystems Synthesizer Model 380A and purified by polyacrylamide gel electrophoresis.

15 Buffers -- PBS is 10 mM sodium phosphate, pH 7.4, 150 mM NaCl. PBB is PBS containing 5 mg/ml bovine serum albumin (BSA) and 2.5 mg/ml bovine gamma globulin. TBS is 50 mM Tris-Cl, pH 7.9, 150 mM NaCl. TBST is TBS containing 0.05% (v/v) Tween[®]-20. SST is
20 15 mM Tris-Cl, pH 7.5, 150 mM NaCl, 2.5 mM EDTA. SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0.

Preparation of cDNA Libraries -- Male term placenta was flash-frozen as 1 cm dice in liquid nitrogen and stored at -70°C. Human umbilical vein
25 endothelial cells were cultured as described by Sadler et al., Proc. Natl. Acad. Sci. 82, 6394-6398 (1985). Total cellular and poly(A)+ RNA was prepared from placenta and endothelial cells according to Chirgwin et al., Biochemistry 18, 5294-5299 (1979), as
30 modified by Sadler et al., supra. Double stranded cDNA was synthesized from both placenta and endothelial cell poly(A)+ RNA according to Gubler and Hoffman, Gene (Amst.) 25, 263-269 (1983). The ends were blunted with mung bean nuclease for 5 min at

25°C in a volume of 200 µl containing 30 mM sodium acetate, pH 4.6, 50 mM NaCl, 1 mM ZnCl₂, 5% (v/v) glycerol, 5.5 µg cDNA, and 150 units mung bean nuclease. Reaction was terminated by
5 phenol/chloroform extraction and ethanol precipitation. The cDNA was then blunted again with T4 DNA polymerase for 30 min at 37°C in a volume of 30 µl containing 33 mM Tris-Cl, pH 8.0, 10 mM magnesium acetate, 66 mM potassium acetate, 1 mM
10 dithiothreitol, 250 µM of each deoxyribonucleotide, 0.1 mg/ml bovine serum albumin, and 3 units T4 DNA polymerase. Reaction was stopped with 1 µl 0.5 M EDTA-Na, pH 8.0, followed by phenol/chloroform extraction and ethanol precipitation. Oligonucleotide
15 adaptors similar to those described by Wood et al., Nature 312, 330-337 (1984), were synthesized with the sequences:

5'-AATTCATCTGTCGACTGCTACC-3'

3'-GTAGACAGCTGACGATGG-5'

20 EcoRI SalI

The shorter oligonucleotide was phosphorylated for 30 min at 37°C in a reaction containing 70 mM Tris-Cl, pH 7.6, 10 mM MgCl₂, 5 mM dithiothreitol, 1 mM ATP, 0.5 units/µl T4 polynucleotide kinase, and 0.1 µg/µl
25 oligonucleotide. After purification by polyacrylamide gel electrophoresis, the phosphorylated short oligonucleotide was annealed to the longer, producing a double stranded adaptor with a blunt end that could be ligated, a cohesive EcoRI

terminus that could not, and a SalI site in the center of the adaptor. Six μg of annealed adaptors were ligated to 2 μg of blunt-ended cDNA in a volume of 20 μl containing 50 mM Tris-Cl, pH 7.6, 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM ATP, and 800 units T4 DNA ligase (New England Biolabs), for 16 h at 14°C. The cDNA was extracted with phenol/chloroform and precipitated with ethanol. Products larger than 1 kb were purified by preparative electrophoresis on a 3.5% polyacrylamide gel and electroelution [Maniatis et al., Mol. Cloning: A Lab. Manual, Cold Spring Harbor Lab., N.Y.(1982)]. The recovered size-selected and adaptor-ligated cDNA was phosphorylated with T4 polynucleotide kinase as described above in a total volume of 20 μl containing 0.3-0.4 μg cDNA. The phosphorylated cDNA was ligated to dephosphorylated $\lambda\text{gt}11$ arms and packaged (Gigapack, Vector Cloning Systems, San Diego, CA). Twenty μg of placental poly(A)+ RNA yielded 0.4 μg of final cDNA that gave 10.5 million independent phage, of which 90% were recombinant. Twenty μg of endothelial cell poly(A)+ RNA yielded 0.6 μg of final cDNA. Packaging of 0.3 μg gave 78.3 million independent phage, of which 92% were recombinant.

25 Isolation of Human Placental PAI,
 Production of Antiserum, and Purification of
 Antibody -- Human placental PAI was purified using an 8-step procedure involving saline extraction, ammonium sulfate fractionation, column chromatography on CM-cellulose *DEAE-Sepharose and hydroxylapatite, chromatofocusing, preparative gel electrophoresis and hydrophobic chromatography, essentially as described by Wun and Reich, J. Biol. Chem. 262(8), 3646-3653 (1987).

A

*Trade mark

Initially, a crude inhibitor fraction was obtained by saline extraction, ammonium sulfate fractionation and CM-cellulose adsorption as described by Holmberg et al., Biochim. Biophys. Acta 544, 128-137 (1978). The crude inhibitor was then subjected to a further 5-step procedure to isolate the inhibitor in essentially homogeneous form as follows:

Step 1: DEAE-Sepharose CL-6B chromatography--

After reduction with dithiothreitol (DTT), the crude inhibitor preparation was adsorbed onto a column of DEAE-Sepharose and chromatographed using a salt gradient with 0 and 0.18 M NaCl as limiting concentrations. Most of the inhibitory activity was eluted as a single peak in the region 0.09-0.13 M NaCl. As measured by the L-pyroglutamyl-glycyl-L-arginine-p-nitroanilide (S2444) amidolytic assay, about 12% of the initial inhibitor was not adsorbed initially and passed directly through the column; this material was not characterized further.

Step 2: Hydroxylapatite chromatography--

The peak fractions from the DEAE-Sepharose column were pooled, dialysed against 0.01 M sodium phosphate buffer, pH 6.8, containing 0.05% β -mercaptoethanol (β ME), and loaded onto a column of hydroxylapatite. After washing the column, the proteins were eluted with a phosphate buffer gradient, pH 6.8, (0.01-0.11 M NaPO_4). Most of the inhibitory material appeared as a single peak which was eluted from the column in the region 0.03-0.07 M NaPO_4 .

Step 3: Chromatofocussing -- The most active fractions obtained from the hydroxylapatite column were pooled, dialysed, and then fractionated by chromatofocussing on *PBE-94 gel (Pharmacia, Inc.)

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*Trade mark

Essentially all of the inhibitory activity was recovered in a single peak in the pH range 5.1-4.6. The elution pH range in this procedure was identical with the pI obtained by isoelectric focussing with
5 ampholines in a flat bed of *Sephadex G-75 gel (data not shown).

Step 4 Preparative polyacrylamide gel electrophoresis--The active material obtained from chromatofocusfocussing was transferred into a
10 dialysis bag which was embedded in Aquacide until enough solvent had been removed to reduce the volume from 10.8 ml to 3 ml. The concentrated solution was then fractionated further by electrophoresis through a polyacrylamide gel column using the Savant
15 preparative gel electrophoresis system. The sample (1.5 ml aliquot) was prepared in 10% glycerol, 20 mM Tris-HCl, pH 8, 0.05% β ME, 20 mM DTT containing tracer bromophenol blue and applied onto a 4-ml column of 10% polyacrylamide gel overlaid with 2 ml of 4%
20 polyacrylamide stacking gel, in a 1 x 11 cm column. The Laemmli gel buffer system, Nature 227, 680-685 (1970), was used except that all buffers contained 0.05% β ME and no SDS was present. Electrophoresis was carried out at room temperature. An initial
25 potential of 75 volts was applied until the protein entered the separating gel. Then the voltage was raised to 125 volts and the proteins were continuously collected from the elution chamber using a peristaltic pump. Fractions of 1.2 ml were
30 collected every 12 min. The position of the inhibitor was located both by inhibition of uPA in the S2444 assay and by analytical SDS-PAGE. The inhibitor was found in fractions 16 to 26 after the appearance of bromophenol blue.

Step 5: Phenyl-Sepharose chromatography --

The inhibitor pool obtained from the preparative PAGE was again concentrated to a final volume of 2 ml by means of Aquacide, dialyzed against
5 10 mM HEPES, pH 8.0, 0.05% β ME, and adsorbed onto a 0.6 x 7 cm phenyl-Sepharose column. The column was then washed with 20 ml of the same buffer at 4°, and the inhibitor was eluted with a buffer containing 50% ethylene glycol, 10 mM HEPES, pH 8.0, and 0.05% β ME
10 at room temperature.

The purified inhibitor had a molecular mass of 47,000 daltons by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [Laemmli, Nature 227, 680-685 (1970)] and formed a 1:1 complex with urokinase.
15 Antiserum against the placental PAI was raised in rabbits by immunization with purified inhibitor. New Zealand white rabbits were treated initially with 100 μ g of purified inhibitor suspended in 2 ml of complete Freund's adjuvant, injected directly into popliteal
20 lymph nodes, subcutaneous sites and the peritoneal cavity. Booster injections of 50 μ g inhibitor in incomplete Freund's adjuvant were administered 5 weeks later. Rabbits were bled weekly beginning at 6 weeks and boosted every month. Specific antibody against
25 the placental inhibitor was purified by affinity chromatography on a placental inhibitor-Sepharose 4B column. The placental inhibitor-Sepharose 4B column was prepared by coupling ~0.8 mg of purified inhibitor to 0.5 g of cyanogen bromide activated Sepharose 4B
30 (Pharmacia) according to the manufacturer's published recommended procedure. Antiserum (3 ml) was mixed with equal volume of PBS supplemented to 0.4 M NaCl, 0.1 M benzamidine, and 1% (v/v) Triton[®] X-100 and applied to the placental inhibitor-Sepharose 4B
35 column. The column was washed with 20 ml of column buffer and then with 20 ml of column buffer without

Triton X-100. Specific antibody was eluted from the column with 0.1 M glycine-HCl, pH 2.2, immediately neutralized with 1/10 volume of 1 M Tris-OH, and dialyzed against 0.15 M NaCl. Each chromatography
5 yielded approximately 2 mg of antibody.

Screening of λ gt11 cDNA Libraries -- Human placenta and endothelial cell cDNA libraries were screened as described by Sadler et al., Proc. Natl. Acad. Sci. USA 82, 6394-6398 (1985), and
10 Young and Davis, Science 222, 778-782 (1983). Affinity-purified rabbit anti-human placental PAI antibody was used at a concentration of 2 μ g/ml in TBST. Bound antibody was detected with horseradish peroxidase conjugated to goat anti-rabbit IgG [deWet
15 et al., DNA(NY) 3, 437-447 (1984)]. Positive isolates were plaque purified and DNA prepared from plate lysates [Helms et al., Ibid. 4, 39-49 (1985)]. The cDNA insert of λ PAI-75.1 was labeled with [α - ^{32} P]-dCTP by nick translation [Maniatis et al., supra.]
20 and employed to screen both cDNA libraries according to Benton and Davis, Science 196, 180-182 (1977). The 14 positives obtained among 300,000 recombinants of the placenta cDNA library were screened with an oligonucleotide corresponding to the complement of
25 λ PAI-75.15 residues -42 to -25. The oligonucleotide was labeled with [γ - ^{32} P]dATP and T4 polynucleotide kinase [Maniatis et al., supra.] to a specific activity of at least 10^8 cpm/ μ g. Filters were prehybridized in 180 mM Tris-Cl, pH 7.5, 0.9 M NaCl,
30 12 mM EDTA, 2 x Denhardt's solution, and 10 μ g/ml denatured salmon sperm DNA. Hybridization was performed in 7 ml of prehybridization buffer per 82 mm filter containing 2.5×10^5 cpm/ml of probe at 46°C for 12 h. The final wash condition was in 2 x
35 SST at 43°C for 20 minutes.

DNA Sequence Analysis -- The cDNA inserts of λ PAI-75.1 and λ PAI-75.15 were subcloned in both orientations into the EcoRI or SalI site of M13mp19 or M13mp18 (cloning vehicles from bacteriophage M13).

5 Deletions were generated using exonuclease III [Henikoff, Gene (Amst.) 28, 357-359(1984)].

Nucleotide sequence was determined by the dideoxy method of Sanger et al., Proc. Natl. Acad. Sci. USA 74, 5463-5467(1977), using [^{35} S]dATP α S and
10 buffer-gradient gels [Biggin et al., Ibid. 80, 3963-3965 (1983)].

Preparation of λ -phage Lysates Containing Recombinant Proteins -- Each plaque purified λ gt11 isolate or λ gt11 was plated with E. coli Y1090 in 8
15 ml LB-0.7% agar onto 15 cm LB-agar plates at a density of 50,000 pfu/plate [Maniatis et al., supra.] Expression of recombinant proteins was induced by isopropyl β -D-thiogalactoside (IPTG) which was added to 1.25 mM in the top agar. After 12 hours
20 at 37°C, 10 ml of TBS was added to each plate and incubated 12 hours at 4°C with gentle agitation. The lysate was recovered and bacterial debris was removed by centrifugation.

Fibrin Autography Assay for PAI Activity
25 of Recombinant proteins -- The λ -lysates containing recombinant proteins were screened for inhibitory activity against urokinase using a fibrin-agar plate spot assay. The fibrin-agar plate was prepared by mixing 2.5 ml of 2 x PBS, 36 μ l of 100 units/ml
30 bovine thrombin, 68 μ l of 3 mg/ml plasminogen, 5.5 ml of 2.5% agarose, and 2.5 ml of 10 mg/ml fibrinogen at 48°C, then pouring the solution onto a 90 mm plastic petri dish. For the spot assay, 45 μ l of λ -lysate was treated with 3 μ l of anti-placental PAI

immunoglobulin (0.5 mg/ml) or 3 μ l of normal rabbit immunoglobulin (0.5 mg/ml) at 4°C for 1 h. Then 5 μ l urokinase solution was added (5 CTA units of Winkinase/ml PBB). After incubation at room temperature for 30 minutes, 2 μ l of the solution was spotted onto the fibrin-agar plate and the plate was incubated in a humidified box at 37°C overnight. Urokinase activity was measured by the appearance of a lytic zone and PAI activity was detected by abolishment of the lytic activity.

SDS-PAGE Assay of Complex Formation between ^{125} I-urokinase and Recombinant Proteins Expressed by λ -phage Isolates -- Aliquots (0.1 ml) of λ -lysates or PBB buffer were mixed with 5 μ l of ^{125} I-urokinase (53 CTA units/ml) and incubated at room temperature for 30 minutes. Three μ l of anti-placental inhibitor IgG (0.2 mg/ml in PBS) was added and the mixture was incubated at 4°C overnight. Twenty-five μ l of goat anti-rabbit immunoglobulin beads (Bio-Rad, each vial was reconstituted in 5 ml of PBB solution) was added and the mixture was incubated for 2.5 hours at room temperature. The beads were collected by centrifugation and washed twice with 1 ml of PBS supplemented to 0.4 M NaCl, 0.1 M benzamidine and 1% (v/v) Triton X-100 and then washed twice with 1 ml of water. Buffer (30 μ l) containing 0.1 M glycine-HCl, pH 2.2, 0.1% (w/v) SDS, 0.001% (w/v) bromphenol blue, 20 mM dithiothreitol and 6 M urea was added to the washed beads and the suspensions were incubated at 37°C for 30 minutes to release the bound material. The suspensions were then centrifuged and 25 μ l of the supernatant was used for SDS-PAGE in a 7.5% polyacrylamide gel [Laemmli, supra.]. The gel was fixed and dried for autoradiography.

Affinity Purification of Antibodies upon

λ gt11 Recombinant Proteins -- This procedure is adapted from Weinberger et al., Science 228, 740-746 (1985). Recombinant proteins from positive λ -phage isolates or from λ gt11 were plated on E. coli Y1090 at 50,000 pfu/15 cm plate of LB-agar in 10 ml LB-0.7% agar and incubated 3 h at 42°C. The soft agar was overlaid with nitrocellulose filters saturated with 10 mM IPTG and incubated at 37°C for 12 h. The filters were removed and incubated in TBST containing 3% (w/v) BSA for 1 h, then incubated with affinity-purified rabbit anti-human placental PAI antibody at a concentration of 2 μ g/ml in TBST at 4°C for 12 hours. Filters were rinsed three times for 5 min each in TBS containing 1 mM EDTA-Na. Bound antibodies were eluted from the filters with two 5 ml aliquots of 4 M $MgCl_2$, 50 mM Tris-Cl, pH 7.5, 0.5% (w/v) BSA, for 5 min each wash. The eluted antibodies were dialyzed against 3 changes of TBST, 1 liter each, at 4°C for 2 hours each change. The dialyzed solutions were used directly to detect purified placenta PAI spotted onto nitrocellulose filters in 1 μ l of TBS containing 0.05 ng, 0.15 ng, 0.5 ng, 2.5 ng, and 5.0 ng PAI protein. Conditions for this assay were as described under Screening of λ gt11 cDNA libraries, above.

Culture of U-937 and HepG2 Cells and

Preparation of RNA -- U-937 cells were grown at 37°C in RPMI supplemented with 10% fetal bovine serum in 150 cm² culture flasks until the cell density reached approximately 1×10^6 cells/ml. The cells were washed twice with PBS and resuspended at a density

of $\sim 2 \times 10^6$ cells/ml in RPMI supplemented with 0.5% (w/v) lactalbumin hydrolysate and 50 U/ml Trasylol, or in the same medium plus 1 μ M PMA. The cells were then incubated further for 18 hours. HepG2 cells
5 were grown at 37°C in DME supplemented with 10% fetal bovine serum in 150 cm² culture flasks to confluency. Poly(A)+ RNA was prepared as described under Preparation of cDNA libraries, above.

Northern Blot Analysis -- Five μ g of each
10 RNA sample was subjected to agarose gel electrophoresis in buffer containing formaldehyde and transferred to nitrocellulose [Maniatis, supra.]. Standards included bovine liver 28S and 18S RNA, and HindIII fragments of λ -phage DNA. The insert of
15 λ PAI-75.1 or a cDNA insert for human γ -actin was labeled by nick translation as described under Screening of λ gt11 cDNA libraries, above, and the blot was hybridized in 50 ml of 50% formamide, 6 x SSC, 25 mM HEPES, pH 7.0, 25 μ g/ml denatured salmon
20 sperm DNA, 1 x Denhardt's solution, 0.1% (w/v) SDS, 1 mM EDTA and 10% dextran sulfate, containing 1×10^7 cpm of probe at 42°C for 16 hours. The filter was washed in 0.1 x SSC, 0.1% SDS at 42°C for 15 minutes, and then at 68°C for 30 minutes. *Kodak XAR-5 film
25 was exposed with the wet filter at -70°C for 10 hours with an intensifying screen.

*Trade mark

A

Protein Sequence Alignment and Computer

Analysis -- The sequences of individual members of the serpin family were aligned by visual inspection and with the assistance of the ALIGN computer program [Dayhoff et al., Methods Enzymol. 91, 524-545 (1983)]. Previously published alignments were also consulted [Bock et al., Biochemistry 25, 4292-4301 (1986), and Carrele et al. in Protease Inhibitors, Barrett and Salvesen, eds., Elsevier/North-Holland Biomed. Press, 1986)]. Phylogeny of sequences was determined by considering the percentage of difference between sequences and alignment scores using either the mutation data matrix or the unitary matrix for the alignment shown in Table I, using the parsimony principle. Sequences amino-terminal to helix A of α_1 -antitrypsin [Loebermann et al., J. Mol. Biol. 177, 531-556 (1984)] were not included in this analysis. The hydropathy or hydrophilicity profiles of placental PAI were calculated using the methods of Hopp and Woods, Proc. Natl. Acad. Sci. USA 78, 3824-3828 (1981), and Kyte and Doolittle, J. Mol. Biol. 157, 105-132 (1982).

The alignment of placental PAI with other serpins is shown in the following Table I.

Table I

ATIII, human:							m
HCII, human:							GSKGPLDQLEKGGETAQSADPOVEQ
Clinh, human:	masrltlltlllllllagdrass	NPNATSSSSQDPESLQDRGEGKVATT	VISKMLFVEPIL				
AGTH, human:							mrkrapqsemapagvslratilcl
AGTH, rat:							mtptgaglkatifci
							1
ECPAI, human:							mqmspaltc
αIAT, human:							mpssvsvgilllaglcclvpvslaEDPQ
αIAT, baboon:							/lllaglccllpgslaEDPQ
αIACT, human:							mermlpllalglilaagfcavilchpNS
ATIII, human:	ysnvigtvtsgkrkvylsllligfwdevtc	HGSPVDICTAKPRDIPMNP	HCIIYRSPEKK				
HCII, human:	LNNKNLSMPLLPADPHKENTVTNDWI	PEGEEDDDYLDLEKIFSEDDDDYIDIVDS	LSVSPT				
Clinh, human:	EVSSLPTTNSTTNSATKITANTTDEPTT	QPTTEPTTQPTIQPTQPTTQLPTDSPT	QPTTG				
AGTH, human:	lavaglaagDRVYIHPFHLVIHNESTCE	QLAKANAGKPKDPTFIPAPIQAKTSPV	DEKAL				
AGTH, rat:	ltvvsitagDRVYIHPFHLVYYSKSTCA	QLENPSVETLPEPTFEPVPIQAKTSPV	DEKTL				
	10	20	30	40	50	60	
		[	helix A	]	[s6B]	[	helix B
PPAI, human:			MEDLCVANTLFALNLFKHLAKASPT-QNL-FLSPWSISSTMA				
Oval, chicken:			mGSIGAASMEFCFDVFKEKLVHVN-ENI-FYCPIAIMSALA				
GeneY, chicken:			MDSISVTNAKFCFDVFNEKVVHVN-ENI-LYCPLSILTALA				
ECPAI, human:	lvlglalvfgegsaVHHPPSYVAHLASDPGVRVFOQVAQASKDR-NV-VFSPYGVASVLA						
αIAT, human:	GDAAQKTDTSHHDDQDHPTFNKITPNLAEFAPSLYRQLAHQSNS-TNI-FFSPVSIATAFA						
αIAT, baboon:	GDAAQKTDTPPHDQNHPTLNKITPSLAEFAPSLYRQLAHQSNS-TNI-FFSPVSIATAFA						
αIACT, human:	PLDEENLTQENQDRGTHVDLGLASANVDFAPSLYKQLVLKALD-KNV-IFSPLSISTALA						
ATIII, human:	ATEDEGSEQKIPEATNRRVWELSKANSRFATTFYQHLADSKNDNDNI-PLSPLSISTAPA						
HCII, human:	DSDVSAGNILQLFHGKSRIQRLNILNAKFAFNLYRVLKDVNTFDNI-FIAPVGISTAMG						
Clinh, human:	SFCPGPVTLCSDLESHSTEAVLGDALVDFSLKLYHAFSAMKKVETNMA-FSPFSIASLLT						
AGTH, human:	QDQLVLVAAKLDTEDKLRAAMVGMLANFLGFRIYGMHSELWGVVBGATVLSPTAVFGTLA						
AGTH, rat:	RDKLVLATEKLEAEDRQRAAQVAMIANFMGFRMYKMLSEARGVASGA-VLSPPALFGTLV						
Consensus:		++	+++	FAF LY+ L		NI F+SP+SI+TALA	
	70	80					
	[	[	helix C	]			[
PPAI, human:	MVYMGSRGSTEDQMAKVLQFNEVGANAVTPMTPENFTSCGFHQIQKGSYPDAILQAQAA						
Oval, chicken:	MVYLGAKDSTRTOINKVVRPDKLP-----GFGDSIEAQCGTS						
GeneY, chicken:	MVYLGARGNTESQMKKVLHFDST-----GAGSTTDSQCGSS						
ECPAI, human:	MLQLTTGGETQOOQQAAMGFKIDD-----KGMA						
αIAT, human:	MLSLGTKADTHDEILEGLNPN-LT-----EIP						
αIAT, baboon:	MLSLGTKADTHSEILEGLNPN-LT-----EIP						
αIACT, human:	FLSLGAHNTTLTEILKASSSP-BG-----DLLR						
ATIII, human:	MTKLGACNDTLQQLMEVFKFDTS-----EKTS						
HCII, human:	MISLGLKGETHEQVHSILHFKDFV-----NASSKYEI						
Clinh, human:	QVLLGAGQNTKTNLESILSY-P-KD-----PTCV						
AGTH, human:	SLYLGAALDHTADRLQAILGVPVKD-----KNCTSRLDAH-KV						
AGTH, rat:	SFYLGSLDPTASQLQVLLGVPVKE-----GDCTSRLDGH-KV						
Consensus:	M++LG+ + T Q+ +L P+						

Table I (cont'd.)

	90	100	110	120	130	140
	helix D]		[sheet 2A]		[helix E][sht 1A]	
PPAI, human:	DKIHSSFRSLSSAINASTGD--YLLESVNKLFGEKSASFREYIRLCQKYYSSE-POAVD					
Oval, chicken:	VNVHSSLRDILNQITKPNDV--YSFSLASRLYAEERYPILPEYLQCVKELYRGG-LEPIN					
GeneY, chicken:	EYVHNLFKELLSEITRPNAT--YSLEIADKLYVDKTFPSVLPEYLSCARKFYTG-VEEVN					
ECPAI, human:	PALRHLYKELMGPWNKD-----EISTTDAIFVQRDLKLVQGFMPHFRLFRST-VKQVD					
α1AT, human:	AQIHEGFQELLRTLNPDSQ--LQLTTDGGFLFLSEGLKLVDFLEDVKKLYHSE-AFTVN					
α1AT, baboon:	AQVHEGFQELLRTLNPDSQ--LQLTTGNGFLFLNLSLVVDKFLLEDVKNLYHSE-AFSVN					
α1ACT, human:	OKFTQSFQHLRAPSISSSDE--LQLSMGNAMFVKEQLSLLDRFTEDAKRLYGSE-AFATD					
ATIII, human:	DQIHFFPAKLNCRLYRKANK-SSKLVSANRLFGDKSLTFNETYQDISELVYGAK-LQPLD					
α2AP, human	/PVS					
HCII, human:	TTIHNLFRKLTHRLFRN--FGYTLRSVNDLYIQKQFPILLDFKTKVREYYPAE-AQIAD					
Clinh, human:	HQALKGFTTKGVTSVSQ-----IFHSPDLAIRDTPVNASRTLYSSS-PR--V					
AGTH, human:	LSALQAVOGLLVAQGRADSQAQLLLSTVVGVTAPGLHLKQPFVQGLALYTPVVLPRSLD					
AGTH, rat:	LTALQAVOGLLVTOGGSSSTPLLQSTVVGFTAPGLRLKQPFVESLGPFTPAIFPRSLD					
Consensus:	+H++F++LL +++ + L + LF + L + + F++ + LY S ++++D					
	150	160	170	180	190	200
	[helix F]		[sheet 3A]			
PPAI, human:	FL-ECAEEARKK-INSWVKTQTKGKIPNLLPEGSVDGDMVLVNAVYFKGKWKTPFEKK					
Oval, chicken:	FQ-TAADQAREL-INSWVESQTNIGIIRNVLOPSSVDSQTAMVLVNAIVFKGLWEKAFKDE					
GeneY, chicken:	FK-TAAEEARQL-INSWVEKETNGQIKDLLVSSSIDFGTTHVFINTIYFKGIWKIAFNTE					
ECPAI, human:	FS-E-VERARFI-INDWVKTHTKGMISNLLGKGAVDQLTRLVLVNALYFNGQWKTPFPDS					
α1AT, human:	FG-D-TEEAKKQ-INDYVEKGTQGKIVDLV--KELDRDTVPALVNYIFFKGKWERPFEVK					
α1AT, baboon:	FE-D-TEEAKKQ-INNYVEKGTQGKVVDLV--KELDRDTVPALVNYIFFKGKWERPFEVE					
α1AT, mouse:	/SPANYILFKGKWKKPFDP					
α1ACT, human:	FQ-D-SAAAKKL-INDYVKNGTRGKITDLI--KDPDSQTMVLVNYIFFKAKWEMPFDPO					
Ctpsn, mouse:	/VVLVNYIYFKGKWKISFDPO					
ATIII, human:	FK-ENAEQSRAA-INKWVSNKTEGRITDVIPSEAINELTVLVLVNTIYFKGLWKSFSPE					
α2AP, human	LT-G-KQEDDLANINQWVKEATEGKIQEF/					
HCII, human:	FS-DPAFISKTN--NHIM-KLTGGLIKDAL--ENIDPATQMMILNCIYFKGSWVNKFPVE					
Clinh, human:	LS-N-NSDANLELINTWVAKNTNNKISRLL--DSLPSDTRLVLLNAIYLSAKWKTTFDPK					
AGTH, human:	F-TE-LDVAAEK-IDRFMQAVTGWKTGCSL--MGASVDSTLAFNTYVHFQGM-KGFSLL					
AGTH, rat:	LSTD-PVLAAQK-INRFVQAVTGWKMNLPL--EGVSTDSTLFFNTYVHFQGM-RGFSQL					
ProZ, barley:	/WV-EQVTXGL-IXEILPP/					
Consensus:	F + + A+ IN+VW+ T+GKI +LL ++D DT +VLVNYIYFKGKW+ +F+ +					
	210	220	230	240	250	
	[sheet 3C]		[sht1B]	[sheet 2B]		[sheet 3B]
PPAI, human:	LNGLYPFRVNSAQRTPVQMMYLREKL-NIGYIEDLK---AQILELPPYA-----GDVSMFL					
Oval, chicken:	DTQAMPFRVTEQESKPVQMMYQIGLF-RVASMASEK---MKILELPPAS-----GTMSMLV					
GeneY, chicken:	DTREMPFSMTKEESKPVQMMCHMNSP-NVATLPAEK---MKILELPPAS-----GDLSMLV					
ECPAI, human:	STHRRLPKSDGSTVSVPMMAQTNKF-NYTEFTTPDGHYYDILELPPYHG-----DTLSMFI					
α1AT, human:	DTEEDDFHVDQVTTVKVPMKRLGMF-NIQHCKKLS---SVLLMKYLG-----NATAIF					
α1AT, baboon:	ATEEDDFHVDQATTVKVPMKRLGMF-NIYHCEKLS---SVLLMKYLG-----NATAIF					
α1AT, mouse:	NTEEAEPHVDSTTVKVPMMTLSGML-DVHHCSTLS---SVLLMDYAG-----NATAVF					
α1ACT, human:	DTHQSRFYLSKKKWMVPMMSLHHLTIPYFRDEELS---CTVVELKYTG-----NASALP					
Ctpsn, mouse:	DTPSEFPYLDEKRSVKVPMKMKLLTTRHFRDEELS---CSVLELKYTG-----NASALL					
ATIII, human:	NTRKELFYKADGESCSASMMYQEGKF-RYRRVAE-G---TQVLELPPKG-----DDITHVL					
α2AP, human	LTQRDSFLHDEQFTVPVEHMQARVYP/					
HCII, human:	MTHNHNFRNLNEREVVKVSMHQTGKNF-LAANDQELD---CDILQLEYVGG-----ISMLI					
Clinh, human:	KTRMEPFHFKNS-VIKVPMHDSKKYPVAHFIDQTLK---AKVGQLQLSH-----NLSLVI					
AGTH, human:	-AEPQEFVVDNSTSVSVPMLSGMGTF--QHWSDIQD---NPSVTQVPFT-----ESACLLL					
AGTH, rat:	-TGLHEFVVDNSTSVSVPMLSGTGNF--QHWSDAQN---NPSVTRVPLG-----ESVTLLL					
ProZ, barley:	N/					
Consensus:	T++ F +++ +V VPMH G F L+ VL+L Y G + S+++					

Table I (cont'd.)

	260	270	280	290	300
	] [helix G] [helix B]		[sht 2C][sheet6A][helix I		
PPAI, human:	LLPDEIADVSTGLELLESEITYDKLNKWT	SKDKMAEDEVEVYIPQFKLEEHYEL	RSILR		
Oval, chicken:	LLPDEV	----GLEQLESIINFEKLTEWTSSNVMEERKIKVYLPRMKMEEKYNL	TSVLM		
GeneY, chicken:	LLPDEV	----GLERIEKTINFDKLREWTSTNAMAKKSMKVYLPRMKIEEKYNL	TSILM		
ECPAI, human:	AAPYEKE	---VPLSALTNILSAQLISHWKGNM	--TRLPRLLVLPKFSLETEVDL	RKPLE	
α 1AT, human:	FLPDEGK	----LQHLENELTHDIITKFLNE	--DRRSASLHLPKLSITGTGYDL	KSVLG	
α 1AT, baboon:	FLPDEGK	----LQHLENELTHDIITKFLNE	--NRRSANLHLPKLAITGTGYDL	KTVLG	
α 1AT, mouse:	LLPDDGK	----MQHLEQTLSEKELISKFLNR	--RRRLAQIHFPRLSISGEYNL	KTLM	
α 1ACT, human:	ILPDQDK	----MEEVEAMLLPETLKRWRDSLEF	REIGELYLPKFSISRDNL	NDILL	
Ctpsn, mouse:	ILPDQGR	----MQQVEASLOPETLRKVRKTLF	PSQIEELNLPKFSIASNYRLEEDVLP		
ATIII, human:	ILPKPEK	----SLAKVEKELTPEVLQEWLDEL	--EEMMLVVMHPRFRIEDGFSL	KEQLQ	
HCII, human:	VVPHKMS	----GMKTLEAQLTPRVVERVQKSM	--TNRTREVLLPKFKLEKNYNL	VESLK	
Clinh, human:	LVPQNLK	---HRLEDMEQALSPSVFKAIHEKLEMSK	-FOPTLLTLPRIKVTTSDMLSIM		
AGTH, human:	IOPHYASD	----LDKVEGLTFQONSLNVMKKL	--SPRTIHLTMPQLVLOGSYDL	QDLLA	
AGTH, rat:	IOPQCAD	----LDRVEVLVFQHDFTVIKNP	--PPRAIRLTLPQLEIRGSYNL	QDLLA	
ProZ, barley:	LLPGAQD	----GLWSLAKRLSTEPEFIENHHPKQTV	EVGRFQLPKFKISYQFEA	SSLLR	
Consensus:	+LPD+	L++LE L+ + + V +	+ R ++ LP++ I	Y+L + +L	
	310	320	330	340	350
	] [sheet 5A] [sheet 4A]				
PPAI, human:	SMGMEDAFNKGRAN	-FSGMSERN	--DLFLSEVFHQAMVDVNEEGTEAAAGTGGVMTG	RT	
Oval, chicken:	AMGITDVFSSS	-AN-LSGISSAE	--SLKISQAVHAAHAEINEAGREVVGSAEAGVDA	AS	
GeneY, chicken:	ALGHTDLFSRS	-AN-LTGISSVD	--NLMSDAVHGVFMEVNEEGTEATGSTGAIGNIKHS		
ECPAI, human:	NLGMTDMFRQFQAD	-FTSLSDQE	--PLHVAQALQKVKIEVNESGTVASSSTAVIVSA	RM	
α 1AT, human:	QLGITKVFSNG	-AD-LSGVTEEA	--PLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIP	MS	
α 1AT, baboon:	HLGITKVFSNG	-AD-LSGVTEEA	--PLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIP	MS	
α 1AT, mouse:	PLGITRIFNNG	-AD-LSGITEENA	-PLKLSQAVHKAVLTIDETGTEAAAVTVLLAVP	YS	
α 1ACT, human:	QLGIEEAFTSK	-AD-LSGITGAR	--NLAVSQVVKVSDVFEEGTEASAATAVKITL	LS	
Ctpsn, mouse:	EMGIKEVFTEQ	-AD-LSGIIETK	--KLSVSQVVKAVLDVAETGTEAAAATGVIGGIRKA		
ATIII, human:	DMGLVDLFSPEKSK	-LPGIVAEGRDDLYVSDAFHKAFLEVNEEGSEAAASTAVVIAG	RS		
HCII, human:	LMGIRMLFDKN	-GNMAGISDORIAIDLK	----HOGTITVNEEGTQATTVTTVGPH	LS	
Clinh, human:	EKLEFFDFS	YD-LN-LCGLTEDP	--DLOVSAMQHQTVLELTETGVEAAAASAI	SV--RT	
AGTH, human:	QAEPLAILTE	-LN-LQKLSNDR	---IRVGEVLNSIFFEL	-EADERE	-PTESTQQ-L-NK
AGTH, rat:	QAKLSTLLGAE	-AN-LGKMGD	TN--P-RVGEVLNSILLEL	-QAGEEEOPTESAQQ	-P-GS
ProZ, barley:	ALGLQLPFSEE	-AD-LSEMVDSS	-QGLEISHVPHKSFVEVNEEGTEAGAATVAMGVA	MS	
Consensus:	+G+ +F+ + A+ LSG+++	L +S+ +HK+++EV+E	GTEA+++T+	+ S	
	360	370	380	390	394
	[sheet 1C] [sheet4B] [sheet 5B]				
PPAI, human:	GHGGPQ	---FVADHPFLFLIMHKITKCILPFGRCSP			
Oval, chicken:	VS-EE	----PRADHPFLPCIKHIATNAVLPFGRCVSP			
GeneY, chicken:	LELEE	----PRADHPFLPFIRYNPTNAILPFGRYWSP			
ECPAI, human:	AP-EE	----IIMDIPFLFVVRHNPTGTVLPHGQVMEP			
α 1AT, human:	IPPE	----VKFNKPFVPLMIEQNTKSPLPHGKVVNPTOK			
α 1AT, baboon:	IPPE	----VKFNKPFVPLMIEQNTKSPLPIGKVVNPTOK			
α 1AT, mouse:	MPPI	----LRFDHPFLPIIFEEHTQSPLFVGKVVDPTHK			
α 1ACT, human:	ALVET	-RTIVRFNRPFPLMIIVPTDTQNI	PFMSKVTNPSKPRACIKQWGSQ		
Ctpsn, mouse:	ILPA	----VHFNRPFPLFVIYHTSAQSILPHAKVNNPK			
ATIII, human:	LNP	N--RVTFKANRPFLVFIREVPLNTIIFMGRVANPCVK			
HCII, human:	-----	TQVRFTVDRPFLFLIYEHR	TSCLLPMGRVANPSRS		
Clinh, human:	LLV	-----FEVQOPFLFVLWDQOHKFPVPMGRVYDPRA			
AGTH, human:	PEVLE	----VTLNRPFPLPAVYDQSATALHPLGRVANPLSTA			
AGTH, rat:	PEVLD	----VTLSSPFLFAIYERDSGALHPLGRVDNPQNVV			
ProZ, barley:	MPLKVDLVD	FVANHPFLFLIREDIAGVVVFVGHVTNPLISA			
Consensus:	+ +++PFLF+I ++ T+++LP+GRV NP				

In the above table, sequences of human antithrombin III (ATIII), human and rat angiotensinogen (AGTH), human and baboon α_1 -antitrypsin (α_1 ACT), ovalbumin (Oval), and chicken gene Y protein (Gene Y) were obtained from the National Biomedical Research Foundation Protein Sequence Database (Georgetown University, Washington, DC, Release 9.0, May 28, 1986), except that threonine was substituted for residue 249 of α_1 -antitrypsin [Kurachi et al., Proc. Natl. Acad. Sci. USA 78, 6826-6830 (1981)]. Sequences of mouse α_1 -antitrypsin and contrapsin (Ctpsn) were translated from the Genbank Genetic Sequence Bank (BBN Laboratories Inc., Cambridge, MA, Release 42.0, May 2, 1986). Endothelial cell PAI-1 (ECPAI), [Ny et al., Proc. Natl. Acad. Sci. USA 83, 6776-6780 (1986), Pannekoek et al., EMBO J. 5, 2539-2544 (1986), and Ginsburg et al., J. Clin. Invest. 78, 1673-1680 (1986)], heparin cofactor II (HCII) [Ragg, Nucleic Acids Res. 14, 1073-1088 (1986)], α_2 -antiplasmin (α_2 AP) [Lijnen et al., Thromb. Haemostasis 48, 311-314 (1982)], Cl-esterase inhibitor (Clnh) [Bock et al., supra] and barley protein Z (ProZ) [Hejgaard et al., FEBS Lett. 180, 89-94 (1985)] were obtained from the directly indicated references. Dashes (---) represent gaps introduced to optimize alignment. Slashes (/) indicate the boundaries of incomplete sequences. The positions of α -helices and the strands of β -sheets are indicated above the aligned sequences according to the crystal structure of α_1 -antitrypsin determined by Loebermann et al., supra, with minor corrections proposed by Bock et al., supra. The limited consensus sequence (Consensus) at the bottom of the alignment was derived as follows:

the residue is listed if present in half or more sequences, a plus (+) is listed if conserved residues are present in two-thirds or more sequences. For this purpose, "conserved" means a positive score in the mutation data matrix [Dayhoff et al., supra]. Sequences are shown in single letter code. Residues removed from the mature proteins are shown in lower case. The arrowheads (►) indicate the active center.

The results of the above laboratory preparative work leading to the complete coding sequence of the cDNA clones representing the full size plasminogen activator inhibitor of the placental type (PAI-2) are further exemplified by the following detailed description of FIGS. 1 to 5 of the drawings.

Initially, 100,000 plaque-forming units from the placenta cDNA library were screened using the affinity purified antibody to human placental PAI. Two positive clones, λ PAI-75.1 and λ PAI-75.2, were found. Screening of the endothelial cell cDNA library yielded four positives among 80,000 recombinants: λ PAI-T, λ PAI-R, λ PAI-89.2 and λ PAI-89.3. The proteins induced by isopropyl- β -D-thiogalactopyranoside from these isolates were screened for PAI activity with a fibrin-agar spot assay (Fig. 1). A standard amount of human u-PA was spotted onto a gel containing fibrin and plasminogen. In the absence of any PAI, the added u-PA activates plasminogen to plasmin, which then degrades the fibrin to produce a cleared lytic zone. In the presence of PAI, lysis is prevented. Inhibition of fibrinolysis was observed only with the protein expressed by isolate λ PAI-75.1. This inhibition was relieved by affinity-purified antibody to placenta PAI, but not by preimmune γ -globulin.

The protein expressed by each of these isolates was also tested for ability to form a covalent complex with u-PA (Fig. 2). Aliquots of λ -phage lysate were incubated with ^{125}I -urokinase (two-chain), and any complexes formed were immunoprecipitated with affinity-purified anti-placental PAI. The immunoprecipitates were subjected to SDS-PAGE after reduction, and the mobility of the ^{125}I -labeled complexes was determined by autoradiography. A complex of $M_r \sim 79,000$ was formed with the protein expressed by $\lambda\text{PAI-75.1}$ (Fig. 2, lane 5). The band at $M_r \sim 20,000$ represents the light chain of two-chain urokinase. The heavy chain of urokinase has $M_r \sim 33,000$ suggesting that the protein expressed by $\lambda\text{PAI-75.1}$ had contributed $M_r \sim 46,000$ to the major complex detected. By Western blotting of $\lambda\text{PAI-75.1}$ lysate proteins, the recombinant PAI was estimated to have $M_r \sim 45,000$, slightly smaller than the natural placental PAI of $M_r \sim 47,000$. This difference may reflect the lack of glycosylation of the protein expressed in E. coli. A faint band at $M_r \sim 88,000$ was detected for isolate $\lambda\text{PAI-89.3}$ (Fig. 2, lane 8). This may represent a distinct urokinase inhibitor which is recognized by the anti-placental PAI antibody. Preliminary sequence data for this cDNA insert shows that it is different from both the endothelial cell PAI and the placental PAI (Fig. 4). A minor band of similar mobility was also seen for $\lambda\text{PAI-75.1}$ (Fig. 2, lane 5).

In the course of cDNA library screening, the strongest signals with the anti-PAI antibody were exhibited by isolates $\lambda\text{PAI-75.1}$ and $\lambda\text{PAI-T}$. The immunological relationship between the proteins expressed by these isolates and authentic placental PAI was tested by the method of "epitope selection",

Weinberger et al., supra. Proteins induced by isopropyl- β -D-thiogalactopyranoside in E. coli infected by these λ -phage or by λ gt11 were bound to nitrocellulose filters and used for affinity
5 purification of the rabbit anti-human PAI antibody. Only isolate λ PAI-75.1 yielded antibody that could recognize natural placental PAI (data not shown), suggesting that the protein expressed by this isolate and placental PAI share at least one epitope.

10 The cDNA insert of λ PAI-75.1 did not cross-hybridize with any of the other positives identified with the antibody to placental PAI, and this insert was used to isolate additional clones by hybridization. Among 300,000 recombinants screened
15 from the endothelial cell cDNA library, no positives were found. Among the same number of recombinants from the placental cDNA library, 15 positives were isolated.

Nucleotide Sequence of Placental PAI cDNA

20 isolates - The cDNA insert of λ PAI-75.1 was 1.9 kb in length and was sequenced by the strategy shown in Fig. 3. The entire sequence was determined on both strands and corresponds to nucleotides -52 to 1829 of Fig. 4, except that nucleotide -52 was G instead of
25 A, and nucleotide 1829 was A instead of C. An oligonucleotide corresponding to the complement of residues -42 to -25 (Fig. 4) was used to screen 14 isolates obtained from the placental cDNA library with the cDNA insert of λ PAI-75.1 as probe. One of
30 these, λ PAI-75.15, was also positive with the oligonucleotide probe. A single sequencing reaction

from each end of this cDNA insert confirmed that
λPAI-75.15 overlapped with λPAI-75.1, with three
additional nucleotides at the 5'-end, and a poly(A)
tail. Although isolated by hybridization, λPAI-
5 75.15 was subsequently shown to express immuno-
reactive PAI of the same size as that of isolate
λPAI-75.1 by Western blotting.

The combined sequence of λPAI-75.1 and
λPAI-75.15 is shown in Fig. 4. The first ATG codon
10 is preceded by a 5'-noncoding region of 55
nucleotides that contains a stop codon in-frame at
residue -27. For isolate λPAI-75.1, this stop codon
is also in-frame with the vector β-galactosidase
coding sequence. The first ATG triplet is followed
15 by an open reading frame of 1245 nucleotides, a stop
codon, a 3'-noncoding region of 581 nucleotides, and
a poly(A) tail. The sequence surrounding the
initiator codon ACAATGG, differs only at position -1
from the proposed optimal sequence for initiation by
20 eukaryotic ribosomes, ACCATGG [Kozak, Cell 44,
283-292 (1986)]. There are five potential
polyadenylation or processing signals with the
sequence AATAAA [Proudfoot and Brownlee, Nature 252,
359-362 (1981)]. Isolates λPAI-75.1 and λPAI-75.15
25 and a third partial cDNA clone (not shown) all share
the same 3' terminus, 25-26 nucleotides after the
pair of overlapping AATAAA sequences at residue 1804.

It will be understood that the "mature"
human placental plasminogen activator inhibitor
30 includes both PAI-2 and methionyl PAI-2 by virtue of
the ATG translational codon in the expression vector
construction herein.

Example 2Materials and Methods

pKK223-3 is an expression vector for regulated overexpression of proteins from cloned
5 genes in E. coli. It contains the strong trp-lac (tac) promoter first described by deBoer et al., Proc. Natl. Acad. Sci. USA 78, 21(1983).

JM 105 is an E. coli K12 lacI^Q host described by Messing, Nucleic Acids Res. 9, 309-321
10 (1981), and Gene 15, 319-329 (1981). In the JM 105 host, the tac promoter is repressed but may be derepressed at the appropriate time by the addition of isopropyl β -thiogalactoside (IPTG).

pUC19 is a small E. coli plasmid cloning
15 vehicle described by Yanich-Perron et al., Gene 33, 103-119 (1985). It consists of parts of pBR322 and M13mp19. pBR322 is the well-known workhorse plasmid of molecular biology described by Bolivar et al., Gene 2, 95 (1977) and Sutcliffe, Nucleic Acids Res.
20 5, 2721-2728 (1978). M13mp19 is a wild type bacteriophage vector suitable for propagation on E. coli JM 105. When the pUC19 plasmid is introduced into JM 105, the plasmid gives rise to blue colonies on appropriate indicator plates. Cloning DNA fragments
25 into any of the multiple restriction sites inactivates the lac gene, giving rise to white colonies.

BPV virus has a genome of 8.0 kilobases. It has the ability to be stably maintained in certain mouse cell lines, e.g. C-127. Foreign DNA can be cloned into the BPV genome and thereby introduced into mouse cells. See J. Virol. 26, 291-298 (1978); Virology 103, 369-375 (1980); and Sarver et al., Mol. Cell. Biol. 1, 486-496 (1981).

C-127 (ATCC CRL 1616) is a nontransformed clonal line derived from a mammary tumor of an RIII mouse.

All the foregoing are well-known, commercially available molecular biologicals.

A. Expression of placental PAI-2 in E. coli

PAI-2 was produced in E. coli strain JM 105 employing a trp-lac promoter vector, pKK 223-3 (obtained from Pharmacia) essentially as described by Amann et al., Gene 25, 167-178 (1983). A purified PAI 75.1/pUC19 plasmid was digested with EcoRI and the PAI 75.1 fragment was inserted into the EcoRI site of the pKK 223-3 vector. The chimeric plasmid was then used to transfect competent JM 105. The transfected cells were grown in the presence of ampicillin. Colonies were randomly picked, grown, and induced by isopropyl β -D-thiogalactoside (IPTG). The cells were lysed by sonication and the lysates were screened for the presence of PA inhibitory activity. As shown in Figure 5, many clones (35% of randomly picked colonies) expressed relatively high level of PAI which inhibits the urokinase induced fibrin lysis in fibrin autography as evident by the non-clearing in 14 wells.

B. Expression of placental PAI-2 in mouse C-127 cells

PAI-2 was also be expressed in a mammalian cell, mouse C-127, employing a bovine papilloma virus (BPV) vector system as described by Howley et al.,
5 Meth. Enzymol. 101, 387-402 (1983). The PAI 75.1 DNA fragment was made blunt ended with the Klenow fragment of DNA polymerase I [Jacobson et al.,
10 Eur. J. Biochem. 45, 623-627 (1974)] by conventional procedure [Maniatis et al., eds., Molecular Cloning: A Laboratory Manual (1982)]. Then a Bam HI linker, d(CGGGATCCCG) was attached by incubation with T4 ligase and the resulting products were digested with BamHI. The Bam HI- PAI 75.1 was then inserted into
15 the Bam HI site of the BPV vector, pMON 1123 (Figure 6). Mouse C-127 cells were then transfected with the chimeric BPV vector essentially as described by Wigler et al., Cell 16, 777-785 (1979), and the transformants were selected for by G418 (genticin) resistance in
20 accordance with methods described by Southern and Berg, J. Molecular Applied Genetics 1, 327-341 (1983). The transfected cells were then grown and the conditioned media were screened for urokinase inhibitory activities using fibrin autography assay similar to that shown in Figure 5. Many clones (~50%
25 of randomly picked colonies) expressed relatively high levels of PAI which inhibits urokinase induced fibrin lysis.

Various other examples will be apparent to the person skilled in the art after reading the
30 present disclosure without departing from the spirit and scope of the invention. All such further examples are included within the scope of the appended claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Human placental plasminogen activator inhibitor cDNA having the nucleotide sequence that follows:

-55 GTTAC CCGTCAGACA GCAACTCAGA
GAATAACCAG AGAACAACCA GATTGAAACA -1

ATG GAG GAT CTT TGT GTG GCA AAC ACA CTC
Met Glu Asp Leu Cys Val Ala Asn Thr Leu
10

TTT GCC CTC AAT TTA TTC AAG CAT CTG GCA
Phe Ala Leu Asn Leu Phe Lys His Leu Ala
20

AAA GCA AGC CCC ACC CAG AAC CTC TTC CTC 90
Lys Ala Ser Pro Thr Gln Asn Leu Phe Leu
30

TCC CCA TGG AGC ATC TCG TCC ACC ATG GCC
Ser Pro Trp Ser Ile Ser Ser Thr Met Ala
40

ATG GTC TAC ATG GGC TCC AGG GGC AGC ACC
Met Val Tyr Met Gly Ser Arg Gly Ser Thr
50

GAA GAC CAG ATG GCC AAG GTG CTT CAG TTT 180
Glu Asp Gln Met Ala Lys Val Leu Gln Phe
60

AAT GAA GTG GGA GCC AAT GCA GTT ACC CCC
Asn Glu Val Gly Ala Asn Ala Val Thr Pro
70

ATG ACT CCA GAG AAC TTT ACC AGC TGT GGG
Met Thr Pro Glu Asn Phe Thr Ser Cys Gly
80

TTC ATG CAG CAG ATC CAG AAG GGT AGT TAT 270
Phe Met Gln Gln Ile Gln Lys Gly Ser Tyr
90

CCT GAT GCG ATT TTG CAG GCA CAA GCT GCA
Pro Asp Ala Ile Leu Gln Ala Gln Ala Ala
100

GAT AAA ATC CAT TCA TCC TTC CGC TCT CTC
Asp Lys Ile His Ser Ser Phe Arg Ser Leu
110

AGC TCT GCA ATC AAT GCA TCC ACA GGG GAT 360
Ser Ser Ala Ile Asn Ala Ser Thr Gly Asp
120

TAT TTA CTG GAA AGT GTC AAT AAG CTG TTT
Tyr Leu Leu Glu Ser Val Asn Lys Leu Phe
130

GGT GAG AAG TCT GCG AGC TTC CGG GAA GAA
Gly Glu Lys Ser Ala Ser Phe Arg Glu Glu
140

TAT ATT CGA CTC TGT CAG AAA TAT TAC TCC 450
Tyr Ile Arg Leu Cys Gln Lys Tyr Tyr Ser
150

TCA GAA CCC CAG GCA GTA GAC TTC CTA GAA
Ser Glu Pro Gln Ala Val Asp Phe Leu Glu
160

TGT GCA GAA GAA GCT AGA AAA AAG ATT AAT
Cys Ala Glu Glu Ala Arg Lys Lys Ile Asn
170

TCC TGG GTC AAG ACT CAA ACC AAA GGC AAA 540
Ser Trp Val Lys Thr Gln Thr Lys Gly Lys
180

ATC CCA AAC TTG TTA CCT GAA GGT TCT GTA
Ile Pro Asn Leu Leu Pro Glu Gly Ser Val
190

GAT GGG GAT ACC AGG ATG GTC CTG GTG AAT
Asp Gly Asp Thr Arg Met Val Leu Val Asn
200

GCT GTC TAC TTC AAA GGA AAG TGG AAA ACT 630
Ala Val Tyr Phe Lys Gly Lys Trp Lys Thr
210

CCA TTT GAG AAG AAA CTA AAT GGG CTT TAT
Pro Phe Glu Lys Lys Leu Asn Gly Leu Tyr
220

CCT TTC CGT GTA AAC TCG GCT CAG CGC ACA
Pro Phe Arg Val Asn Ser Ala Gln Arg Thr
230

CCT GTA CAG ATG ATG TAC TTG CGT GAA AAG 720
Pro Val Gln Met Met Tyr Leu Arg Glu Lys
240

CTA AAC ATT GGA TAC ATA GAA GAC CTA AAG
Leu Asn Ile Gly Tyr Ile Glu Asp Leu Lys
250

GCT CAG ATT CTA GAA CTC CCA TAT GCT GGA
Ala Gln Ile Leu Glu Leu Pro Tyr Ala Gly
260

GAT GTT AGC ATG TTC TTG TTG CTT CCA GAT 810
Asp Val Ser Met Phe Leu Leu Leu Pro Asp
270

GAA ATT GCC GAT GTG TCC ACT GGC TTG GAG
Glu Ile Ala Asp Val Ser Thr Gly Leu Glu
280

CTG CTG GAA AGT GAA ATA ACC TAT GAC AAA
Leu Leu Glu Ser Glu Ile Thr Tyr Asp Lys
290

CTC AAC AAG TGG ACC AGC AAA GAC AAA ATG 900
Leu Asn Lys Trp Thr Ser Lys Asp Lys Met
300

GCT GAA GAT GAA GTT GAG GTA TAC ATA CCC Ala Glu Asp Glu Val Glu Val Tyr Ile Pro 310	TGCCTATTTA ATGTAGCTAA TAAAGTTATA GAAGCAGATG ATCTGTTAAT TTCCTATCTA ATAAATGCCT TTAATTGTTT TCATAATGAA GAATAAGTAG GTATCCCTCC ATGCCCTTCT ATAATAAATA TCTGGAAAAA ACATTAAACA ATAGGCAAAT ATATGTTATG TGCATTCTA GAAATACATA ACACATATAT ATGTCTGTAT CTTATATTCA ATTGCAAGTA TATAATAAAT AAACCTGCTT CCAAACAACA AAAAAAAAAA AAAAAAAAA AAAAA.	1590 1700 1810 1855
CAG TTC AAA TTA GAA GAG CAT TAT GAA CTC Gln Phe Lys Leu Glu Glu His Tyr Glu Leu 320		
AGA TCC ATT CTG AGA AGC ATG GGC ATG GAG Arg Ser Ile Leu Arg Ser Met Gly Met Glu 330		
GAC GCC TTC AAC AAG GGA CGG GCC AAT TTC Asp Ala Phe Asn Lys Gly Arg Ala Asn Phe 340		
TCA GGG ATG TCG GAG AGG AAT GAC CTG TTT Ser Gly Met Ser Glu Arg Asn Asp Leu Phe 350		
CTT TCT GAA GTG TTC CAC CAA GCC ATG GTG Leu Ser Glu Val Phe His Gln Ala Met Val 360		
GAT GTG AAT GAG GAG GGC ACT GAA GCA GCC Asp Val Asn Glu Glu Gly Thr Glu Ala Ala 370		
GCT GGC ACA GGA GGT GTT ATG ACA GGG AGA Ala Gly Thr Gly Gly Val Met Thr Gly Arg 380		
ACT GGA CAT GGA GGC CCA CAG TTT GTG GCA Thr Gly His Gly Gly Pro Gln Phe Val Ala 390		
GAT CAT CCG TTT CTT TTT CTT ATT ATG CAT Asp His Pro Phe Leu Phe Leu Ile Met His 400		
AAG ATA ACC AAG TGC ATT TTA TTT TTC GGC Lys Ile Thr Lys Cys Ile Leu Phe Phe Gly 410		
AGA TTT TGC TCA CCC TAA AAC TAA GCG TGC Arg Phe Cys Ser Pro End		
TGCTTCTGCA AAAGATTTTT GTAGATGAGC TGTGTGCCTC AGAATTGCTA TTTCAAATTG CCAAAAATTT AGAGATGTTT TCTACATATT TCTGCTCTTC TGAACAACCT CTGCTACCCA CTAAATAAAA ACACAGAAAT AATTAGACAA TTGTCTATTA TAACATGACA ACCCTATTAA TCATTTGGTC TTCTAAAATG GGATCATGCC CATTTAGATT TTCCTTACTA TCAGTTTATT TTTATAACAT TAACTTTTAC TTTGTTATTT ATTATTTTAT ATAATGGTGA GTTTTAAAT TATTGCTCAC	1370 1480	

2. Human placental plasminogen activator inhibitor cDNA
having the nucleotide sequence that follows:

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AC CCGTCAGACA GCAACTCAGA GAATAACCAG AGAACAACCA GATTGAAACA   -1

ATG GAG GAT CTT TGT GTG GCA AAC ACA CTC
Met Glu Asp Leu Cys Val Ala Asn Thr Leu
                                     10

TTT GCC CTC AAT TTA TTC AAG CAT CTG GCA
Phe Ala Leu Asn Leu Phe Lys His Leu Ala
                                     20

AAA GCA AGC CCC ACC CAG AAC CTC TTC CTC
Lys Ala Ser Pro Thr Gln Asn Leu Phe Leu
                                     30
                                     90

TCC CCA TGG AGC ATC TCG TCC ACC ATG GCC
Ser Pro Trp Ser Ile Ser Ser Thr Met Ala
                                     40

ATG GTC TAC ATG GGC TCC AGG GGC AGC ACC
Met Val Tyr Met Gly Ser Arg Gly Ser Thr
                                     50

GAA GAC CAG ATG GCC AAG GTG CTT CAG TTT
Glu Asp Gln Met Ala Lys Val Leu Gln Phe
                                     60
                                     180

AAT GAA GTG GGA GCC AAT GCA GTT ACC CCC
Asn Glu Val Gly Ala Asn Ala Val Thr Pro
                                     70

ATG ACT CCA GAG AAC TTT ACC AGC TGT GGG
Met Thr Pro Glu Asn Phe Thr Ser Cys Gly
                                     80

TTC ATG CAG CAG ATC CAG AAG GGT AGT TAT
Phe Met Gln Gln Ile Gln Lys Gly Ser Tyr
                                     90
                                     270

CCT GAT GGC ATT TTG CAG GCA CAA GCT GCA
Pro Asp Ala Ile Leu Gln Ala Gln Ala Ala
                                     100

GAT AAA ATC CAT TCA TCC TTC CGC TCT CTC
Asp Lys Ile His Ser Ser Phe Arg Ser Leu
                                     110

AGC TCT GCA ATC AAT GCA TCC ACA GGG GAT
Ser Ser Ala Ile Asn Ala Ser Thr Gly Asp
                                     120
                                     360

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TAT TTA CTG GAA AGT GTC AAT AAG CTG TTT
Tyr Leu Leu Glu Ser Val Asn Lys Leu Phe
130

GGT GAG AAG TCT GCG AGC TTC CGG GAA GAA
Gly Glu Lys Ser Ala Ser Phe Arg Glu Glu
140

TAT ATT CGA CTC TGT CAG AAA TAT TAC TCC
Tyr Ile Arg Leu Cys Gln Lys Tyr Tyr Ser
150 450

TCA GAA CCC CAG GCA GTA GAC TTC CTA GAA
Ser Glu Pro Gln Ala Val Asp Phe Leu Glu
160

TGT GCA GAA GAA GCT AGA AAA AAG ATT AAT
Cys Ala Glu Glu Ala Arg Lys Lys Ile Asn
170

TCC TGG GTC AAG ACT CAA ACC AAA GGC AAA
Ser Trp Val Lys Thr Gln Thr Lys Gly Lys
180 540

ATC CCA AAC TTG TTA CCT GAA GGT TCT GTA
Ile Pro Asn Leu Leu Pro Glu Gly Ser Val
190

GAT GGG GAT ACC AGG ATG GTC CTG GTG AAT
Asp Gly Asp Thr Arg Met Val Leu Val Asn
200

GCT GTC TAC TTC AAA GGA AAG TGG AAA ACT
Ala Val Tyr Phe Lys Gly Lys Trp Lys Thr
210 630

CCA TTT GAG AAG AAA CTA AAT GGG CTT TAT
Pro Phe Glu Lys Lys Leu Asn Gly Leu Tyr
220

CCT TTC CGT GTA AAC TCG GCT CAG CGC ACA
Pro Phe Arg Val Asn Ser Ala Gln Arg Thr
230

CCT GTA CAG ATG ATG TAC TTG CGT GAA AAG
Pro Val Gln Met Met Tyr Leu Arg Glu Lys
240 720

CTA AAC ATT GGA TAC ATA GAA GAC CTA AAG
Leu Asn Ile Gly Tyr Ile Glu Asp Leu Lys
250

GCT CAG ATT CTA GAA CTC CCA TAT GCT GGA
Ala Gln Ile Leu Glu Leu Pro Tyr Ala Gly
260

GAT GTT AGC ATG TTC TTG TTG CTT CCA GAT
Asp Val Ser Met Phe Leu Leu Leu Pro Asp
270

810

GAA ATT GCC GAT GTG TCC ACT GGC TTG GAG
Glu Ile Ala Asp Val Ser Thr Gly Leu Glu
280

CTG CTG GAA AGT GAA ATA ACC TAT GAC AAA
Leu Leu Glu Ser Glu Ile Thr Tyr Asp Lys
290

CTC AAC AAG TGG ACC AGC AAA GAC AAA ATG
Leu Asn Lys Trp Thr Ser Lys Asp Lys Met
300

900

GCT GAA GAT GAA GTT GAG GTA TAC ATA CCC
Ala Glu Asp Glu Val Glu Val Tyr Ile Pro
310

CAG TTC AAA TTA GAA GAG CAT TAT GAA CTC
Gln Phe Lys Leu Glu Glu His Tyr Glu Leu
320

AGA TCC ATT CTG AGA AGC ATG GGC ATG GAG
Arg Ser Ile Leu Arg Ser Met Gly Met Glu
330

990

GAC GCC TTC AAC AAG GGA CGG GCC AAT TTC
Asp Ala Phe Asn Lys Gly Arg Ala Asn Phe
340

TCA GGG ATG TCG GAG AGG AAT GAC CTG TTT
Ser Gly Met Ser Glu Arg Asn Asp Leu Phe
350

CTT TCT GAA GTG TTC CAC CAA GCC ATG GTG
Leu Ser Glu Val Phe His Gln Ala Met Val
360

1080

GAT GTG AAT GAG GAG GGC ACT GAA GCA GCC
Asp Val Asn Glu Glu Gly Thr Glu Ala Ala
370

GCT GGC ACA GGA GGT GTT ATG ACA GGG AGA
Ala Gly Thr Gly Gly Val Met Thr Gly Arg
380

ACT GGA CAT GGA GGC CCA CAG TTT GTG GCA 1170
 Thr Gly His Gly Gly Pro Gln Phe Val Ala
 390

GAT CAT CCG TTT CTT TTT CTT ATT ATG CAT
 Asp His Pro Phe Leu Phe Leu Ile Met His
 400

AAG ATA ACC AAG TGC ATT TTA TTT TTC GGC
 Lys Ile Thr Lys Cys Ile Leu Phe Phe Gly
 410

AGA TTT TGC TCA CCC TAA AAC TAA GCG TGC 1260
 Arg Phe Cys Ser Pro End

TGCTTCTGCA AAAGATTTTT GTAGATGAGC TGTGTGCCTC AGAATTGCTA TTTCAAATTG 1320

CCAAAAATTT AGAGATGTTT TCTACATATT TCTGCTCTTC TGAACAACTT CTGCTACCCA 1380

CTAAATAAAA ACACAGAAAT AATTAGACAA TTGTCTATTA TAACATGACA ACCCTATTAA 1440

TCATTTGGTC TTCTAAAATG GGATCATGCC CATTAGATT TTCCTTACTA TCAGTTTATT 1500

TTTATAACAT TAACTTTTAC TTTGTTATTT ATTATTTAT ATAATGGTGA GTTTTTAAAT 1560

TATTGCTCAC TGCCTATTTA ATGTAGCTAA TAAAGTTATA GAAGCAGATG ATCTGTTAAT 1620

TTCCTATCTA ATAAATGCCT TTAATTGTTC TCATAATGAA GAATAAGTAG GTATCCCTCC 1680

ATGCCCTTCT ATAATAAATA TCTGGAAAAA ACATTAAACA ATAGGCAAAT ATATGTTATG 1740

TGCATTTICTA GAAATACTA ACACATATAT ATGTCTGTAT CTTATATTCA ATTGCAAGTA 1800

TATAATAAAT AAACCTGCTT CCAAACAAC

3. A recombinant DNA sequence comprising a sequence encoding human placental plasminogen activator inhibitor, said human placental plasminogen activator inhibitor having the amino acid sequence that follows:

Met Glu Asp Leu Cys Val Ala Asn Thr Leu Phe Ala Leu Asn Leu Phe Lys His Leu Ala Lys Ala Ser Pro Thr Gln Asn Leu Phe Leu
10 20 30
Ser Pro Trp Ser Ile Ser Ser Thr Met Ala Met Val Tyr Met Gly Ser Arg Gly Ser Thr Glu Asp Gln Met Ala Lys Val Leu Gln Phe
40 50 60
Asn Glu Val Gly Ala Asn Ala Val Thr Pro Met Thr Pro Glu Asn Phe Thr Ser Cys Gly Phe Met Gln Gln Ile Gln Lys Gly Ser Tyr
70 80 90
Pro Asp Ala Ile Leu Gln Ala Gln Ala Ala Asp Lys Ile His Ser Ser Phe Arg Ser Leu Ser Ser Ala Ile Asn Ala Ser Thr Gly Asp
100 110 120
Tyr Leu Leu Glu Ser Val Asn Lys Leu Phe Gly Glu Lys Ser Ala Ser Phe Arg Glu Glu Tyr Ile Arg Leu Cys Gln Lys Tyr Tyr Ser
130 140 150
Ser Glu Pro Gln Ala Val Asp Phe Leu Glu Cys Ala Glu Glu Ala Arg Lys Lys Ile Asn Ser Trp Val Lys Thr Gln Thr Lys Gly Lys
160 170 180
Ile Pro Asn Leu Leu Pro Glu Gly Ser Val Asp Gly Asp Thr Arg Met Val Leu Val Asn Ala Val Tyr Phe Lys Gly Lys Trp Lys Thr
190 200 210
Pro Phe Glu Lys Lys Leu Asn Gly Leu Tyr Pro Phe Arg Val Asn Ser Ala Gln Arg Thr Pro Val Gln Met Met Tyr Leu Arg Glu Lys
220 230 240
Leu Asn Ile Gly Tyr Ile Glu Asp Leu Lys Ala Gln Ile Leu Glu Leu Pro Tyr Ala Gly Asp Val Ser Met Phe Leu Leu Leu Pro Asp
250 260 270
Glu Ile Ala Asp Val Ser Thr Gly Leu Glu Leu Leu Glu Ser Glu Ile Thr Tyr Asp Lys Leu Asn Lys Trp Thr Ser Lys Asp Lys Met
280 290 300
Ala Glu Asp Glu Val Glu Val Tyr Ile Pro Gln Phe Lys Leu Glu Glu His Tyr Glu Leu Arg Ser Ile Leu Arg Ser Met Gly Met Glu
310 320 330
Asp Ala Phe Asn Lys Gly Arg Ala Asn Phe Ser Gly Met Ser Glu Arg Asn Asp Leu Phe Leu Ser Glu Val Phe His Gln Ala Met Val
340 350 360
Asp Val Asn Glu Glu Gly Thr Glu Ala Ala Ala Gly Thr Gly Gly Val Met Thr Gly Arg Thr Gly His Gly Gly Pro Gln Phe Val Ala
370 380 390
Asp His Pro Phe Leu Phe Leu Ile Met His Lys Ile Thr Lys Cys Ile Leu Phe Phe Gly Arg Phe Cys Ser Pro.
400 410

4. A replicable expression vector comprising the DNA sequence of Claim 3.

5. A viable cell culture transfected with the expression vector of Claim 4.

6. A bacterial culture transformed with the expression vector of claim 4 capable of producing mature human placental plasminogen activator inhibitor.

7. A mammalian cell culture transformed with the expression vector of claim 4 capable of producing mature human placental plasminogen activator inhibitor.

8. A process which comprises expressing a gene encoding human placental plasminogen activator inhibitor having the amino acid sequence that follows:

Met	Glu	Asp	Leu	Cys	Val	Ala	Asn	Thr	Leu	10	Leu	Asn	Ile	Gly	Tyr	Ile	Glu	Asp	Leu	Lys	250
Phe	Ala	Leu	Asn	Leu	Phe	Lys	His	Leu	Ala	20	Ala	Gln	Ile	Leu	Glu	Leu	Pro	Tyr	Ala	Gly	260
Lys	Ala	Ser	Pro	Thr	Gln	Asn	Leu	Phe	Leu	30	Asp	Val	Ser	Met	Phe	Leu	Leu	Leu	Pro	Arg	270
Ser	Pro	Trp	Ser	Ile	Ser	Ser	Thr	Met	Ala	40	Glu	Ile	Ala	Asp	Val	Ser	Thr	Gly	Leu	Glu	280
Met	Val	Tyr	Met	Gly	Ser	Arg	Gly	Ser	Thr	50	Leu	Leu	Glu	Ser	Glu	Ile	Thr	Tyr	Asp	Lys	290
Glu	Asp	Gln	Met	Ala	Lys	Val	Leu	Gln	Phe	60	Leu	Asn	Lys	Trp	Thr	Ser	Lys	Asp	Lys	Met	300
Asn	Glu	Val	Gly	Ala	Asn	Ala	Val	Thr	Pro	70	Ala	Glu	Asp	Glu	Val	Glu	Val	Tyr	Ile	Pro	310
Met	Thr	Pro	Glu	Asn	Phe	Thr	Ser	Cys	Gly	80	Gln	Phe	Lys	Leu	Glu	Glu	His	Tyr	Glu	Leu	320
Phe	Met	Gln	Gln	Ile	Gln	Lys	Gly	Ser	Tyr	90	Arg	Ser	Ile	Leu	Arg	Ser	Met	Gly	Met	Glu	330
Pro	Asp	Ala	Ile	Leu	Gln	Ala	Gln	Ala	Ala	100	Asp	Ala	Phe	Asn	Lys	Gly	Arg	Ala	Asn	Phe	340
Asp	Lys	Ile	His	Ser	Ser	Phe	Arg	Ser	Leu	110	Ser	Gly	Met	Ser	Glu	Arg	Asn	Asp	Leu	Phe	350
Ser	Ser	Ala	Ile	Asn	Ala	Ser	Thr	Gly	Asp	120	Leu	Ser	Glu	Val	Phe	His	Gln	Ala	Met	Val	360
Tyr	Leu	Leu	Glu	Ser	Val	Asn	Lys	Leu	Phe	130	Asp	Val	Asn	Glu	Glu	Gly	Thr	Glu	Ala	Ala	370
Gly	Glu	Lys	Ser	Ala	Ser	Phe	Arg	Glu	Glu	140	Ala	Gly	Thr	Gly	Gly	Val	Met	Thr	Gly	Arg	380
Tyr	Ile	Arg	Leu	Cys	Gln	Lys	Tyr	Tyr	Ser	150	Thr	Gly	His	Gly	Gly	Pro	Gln	Phe	Val	Ala	390
Ser	Glu	Pro	Gln	Ala	Val	Asp	Phe	Leu	Glu	160	Asp	His	Pro	Phe	Leu	Phe	Leu	Ile	Met	His	400
Cys	Ala	Glu	Glu	Ala	Arg	Lys	Lys	Ile	Asn	170	Lys	Ile	Thr	Lys	Cys	Ile	Leu	Phe	Phe	Gly	410
Ser	Trp	Val	Lys	Thr	Gln	Thr	Lys	Gly	Lys	180	Arg	Phe	Cys	Ser	Pro						
Ile	Pro	Asn	Leu	Leu	Pro	Glu	Gly	Ser	Val	190											
Asp	Gly	Asp	Thr	Arg	Met	Val	Leu	Val	Asn	200											
Ala	Val	Tyr	Phe	Lys	Gly	Lys	Trp	Lys	Thr	210											
Pro	Phe	Glu	Lys	Lys	Leu	Asn	Gly	Leu	Tyr	220											
Pro	Phe	Arg	Val	Asn	Ser	Ala	Gln	Arg	Thr	230											
Pro	Val	Gln	Met	Met	Tyr	Leu	Arg	Glu	Lys	240											

in a bacterial or mammalian cell culture transformed with a DNA expression vector containing said gene operably linked to transcription and translation sequences in said vector and recovering said human placental plasminogen activator inhibitor.

1/5

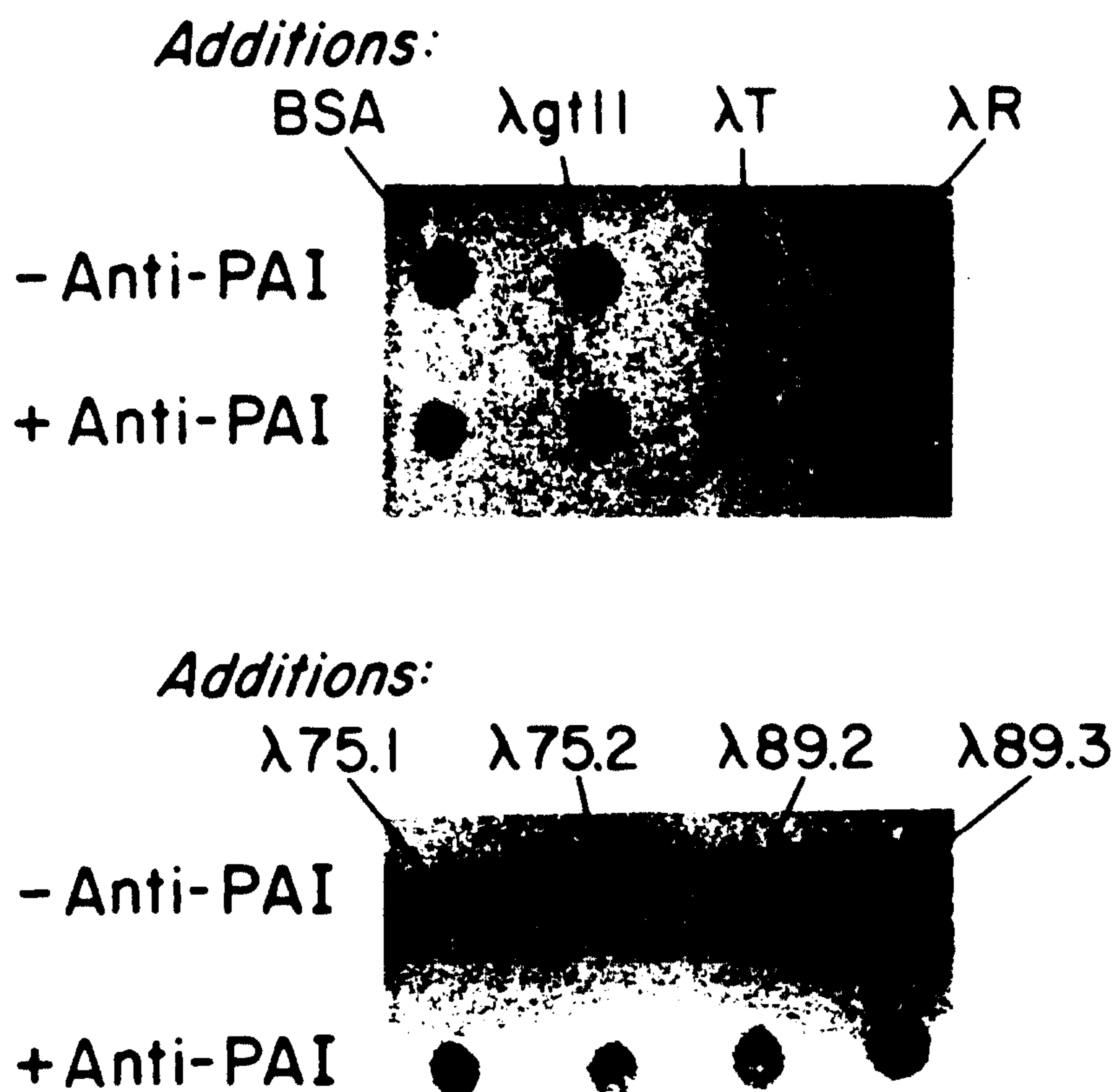


FIG. 1.

MW $\times 10^{-3}$	1	2	3	4	5	6	7	8
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88—
79—

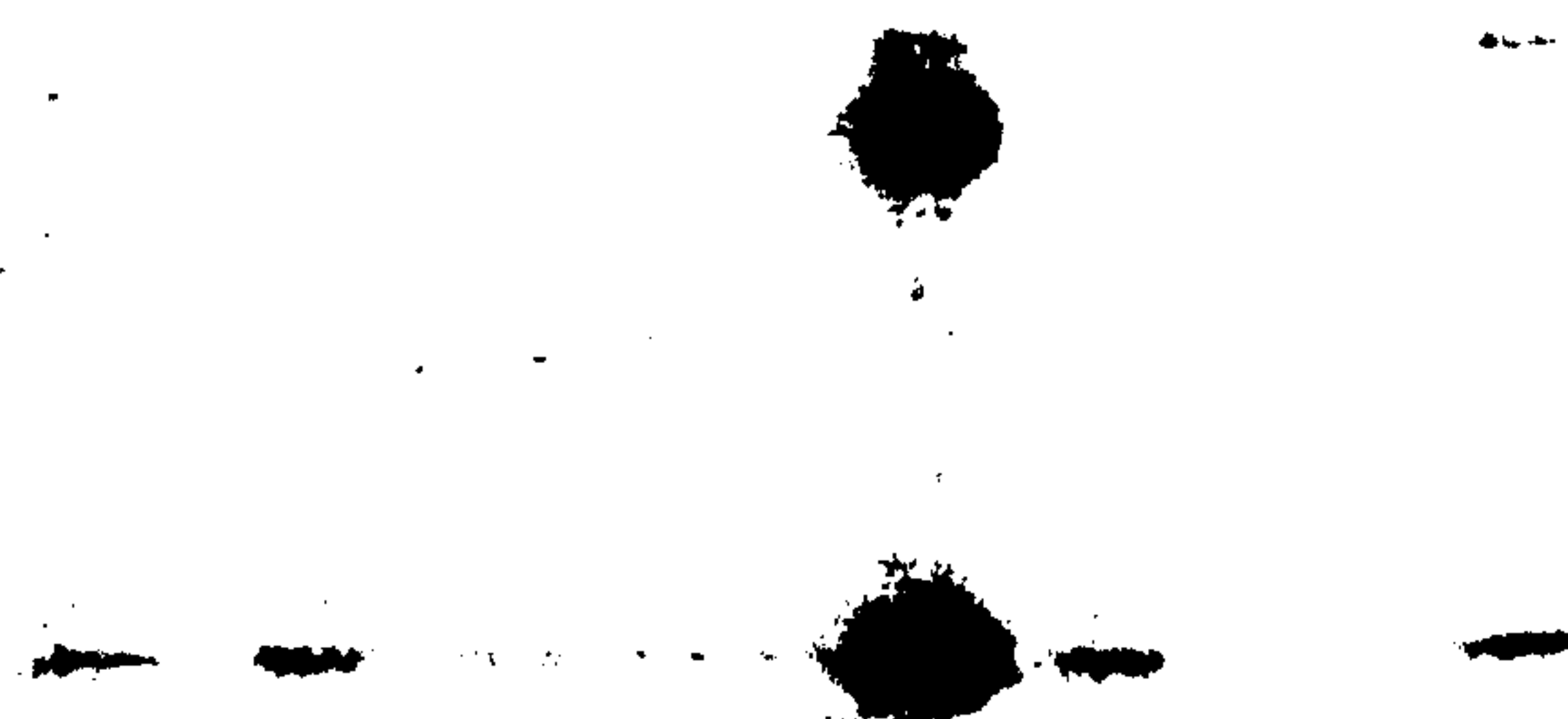


FIG. 2.

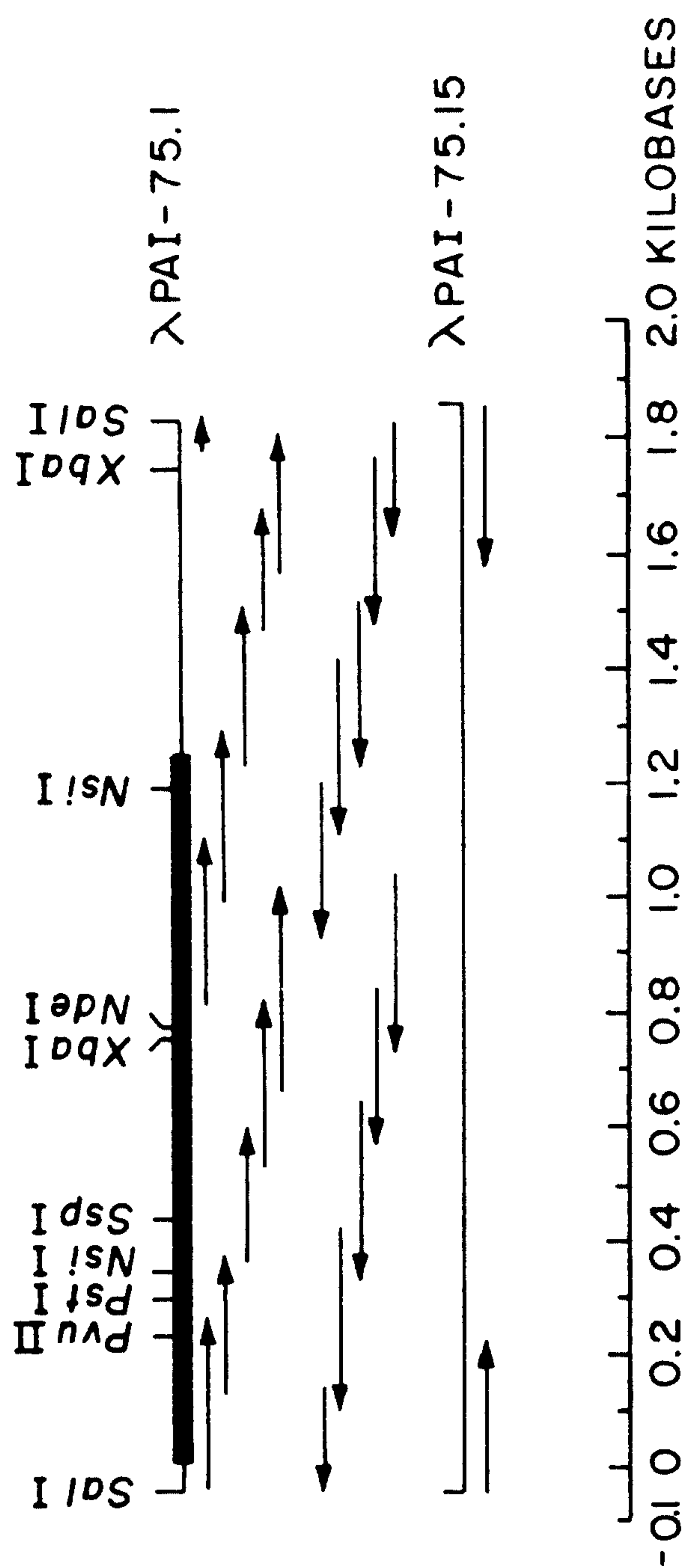


FIG. 3.

1 3 4 1 3 1 8

3/5

-1
-55 GTTAC CCGTCAGACA GCAACTCAGA GAATAACCA AGAACACCA GATTGAAACA
ATG GAG GAT CTT TGT GTG GCA AAC ACA CTC TTT GCC CTC AAT TTA TTC AAG CAT CTG GCA AAA GCA AGC CCC ACC CAG AAC CTC TTC CTC
Met Glu Asp Leu Cys Val Ala Asn Thr Leu Phe Ala Leu Asn Thr Leu Phe Lys His Leu Ala Lys Ala Ser Pro Thr Gln Asn Leu Phe Leu
10 20 30
TCC CCA TGG AGC ATC TCG TCC ACC ATG GGC ATG GTC TAC ATG GGC TCC AGG GGC AGC ACC GAA GAC CAG ATG GCC AAG GTG CTT CAG TTT
Ser Pro Trp Ser Ile Ser Ser Thr Met Ala Met Val Tyr Met Gly Ser Arg Gly Ser Thr Glu Asp Gln Met Ala Lys Val Leu Gln Phe
40 50 60
AAT GAA GTG GGA GCC AAT GCA GTT ACC CCC ATG ACC ATG CCC ATG GGC TCC AGG GGC AGC AGC GAA GGC TCC AGG GGC TCC AGG GGC AGC ACC GAA GAC CAG ATG GCC AAG GTG CTT CAG TTT
Asn Glu Val Gly Ala Asn Ala Val Thr Pro Met Thr Pro Glu Asn Phe Thr Ser Cys Gly Phe Met Gln Gln Ile Gln Lys Gly Ser Tyr
70 80 90
CCT GAT GCG ATT TTG CAG GCA CAA GCT GCA GAT AAA ATC CAT TCA TCC TTC CGC TCT CTC AGC TCT GCA ATC AAT GCA TCC ACA GGG GAT
Pro Asp Ala Ile Leu Gln Ala Gln Ala Ala Asp Lys Ile His Ser Ser Phe Arg Ser Leu Ser Ser Ala Ile Asn Ala Ser Thr Gly Asp
100 110 120
TAT TTA CTG GAA AGT GTC AAT AAG CTG TTT GGT GAG AAG TCT GCG AGC TTC CGG GAA GAA TAT ATT CGA CTC TGT CAG AAA TAT TAC TCC
Tyr Leu Leu Glu Ser Val Asn Lys Leu Phe Phe Gly Glu Lys Ser Ala Ser Phe Arg Glu Glu Gln Tyr Ile Arg Leu Cys Gln Lys Tyr Tyr Ser
130 140 150
TCA GAA CCC CAG GCA GTA GAC TTC CTA GAA TGT GCA GAA GAA GCT AGA AAA AAG ATT AAT TCC TGG GTC AAG ACT CAA ACC AAA GGC AAA
Ser Glu Pro Gln Ala Val Asp Phe Leu Leu Cys Ala Glu Glu Ala Arg Lys Lys Ile Asn Ser Trp Val Lys Thr Gln Thr Lys Gly Lys
160 170 180
ATC CCA AAC TTG TTA CCT GAA GGT TCT GTA GAT GGG GAT ACC AGG ATG GTC CTG GTG AAT GCT GTC TAC TTC AAA GGA AAG TGG AAA ACT
Ile Pro Asn Leu Leu Leu Pro Glu Gly Ser Val Asp Gly Asp Thr Arg Met Val Leu Val Asn Ala Val Tyr Phe Lys Gly Lys Trp Lys
190 200 210
CCA TTT GAG AAG AAA CTA AAT GGG CTT TAT CCT TTC COT GTA AAC TCG GCT CAG CGC ACA CCT GTA CAG ATG ATG TAC TTG CGT GAA AAG
Pro Phe Glu Lys Lys Leu Asn Gly Leu Tyr Pro Phe Arg Val Asn Ser Ala Gln Arg Thr Pro Val Gln Met Met Tyr Leu Arg Glu Lys
220 230 240

FIG. 4A.

CTA AAC ATT GGA TAC ATA GAA GAC CTA AAG GCT CAG ATT CTA GAA CTC CCA TAT GCT GGA GAT GTT AGC ATG TTC TTG TTG CTT CCA GAT 810
Leu Asn Ile Gly Tyr Ile Glu Asp Leu Lys Ala Glu Ile Leu Glu Leu Pro Tyr Ala Gly Asp Val Ser Met Phe Leu Leu Leu Pro Asp 270
250
GAA ATT GCC GAT GTG TCC ACT GGC TCG GAG CTG CTG GAA AGT GAA ATA ACC TAT GAC AAA CTC AAC AAG TGG ACC AGC AAA GAC AAA ATG 900
Glu Ile Ala Asp Val Ser Thr Gly Leu Glu Leu Leu Glu Ser Glu Ile Thr Tyr Asp Lys Leu Asn Lys Trp Thr Ser Lys Asp Lys Met 300
280
GCT GAA GAT GAA GTT GAG GTA TAC ATA CCC CAG TTC AAA TTA GAA GAG CAT TAT GAA CTC AGA TCC ATT CTG AGA AGC ATG GGC ATG GAG 990
Ala Glu Asp Glu Val Glu Val Tyr Ile Pro Glu Phe Lys Leu Glu Glu His Tyr Glu Leu Arg Ser Ile Leu Arg Ser Met Gly Met Glu 330
310
GAC GCC TTC AAC AAG GGA CGG GCC AAT TTC TCA GGG ATG TCG GAG AGG AAT GAC CTG TTT CTT TCT GAA GTG TTC CAC CAA GCC ATG GTG 1080
Asp Ala Phe Asn Lys Gly Arg Ala Asn Phe Ser Gly Met Ser Glu Arg Asn Asp Leu Phe Leu Ser Glu Val Phe His Gln Ala Met Val 360
340
GAT GTG AAT GAG GAG GGC ACT GAA GCA GCC GCT GCT GGC ACA GGA GGT GTT ATG ACA GGG AGA ACT GGA CAT GGA GGC CCA CAG TTT GTG GCA 1170
Asp Val Asn Glu Glu Gly Thr Glu Ala Glu Thr Gly Gly Val Met Thr Gly Arg Thr Gly His Gly Gly Pro Gln Phe Val Ala 390
370
GAT CAT CCG TTT CTT TTT CTT ATT ATG CAT AAG ATA ACC AAG TGC ATT TTA TTT TTC GGC AGA TTT TGC TCA CCC TAA AAC TAA GCG TGC 1260
Asp His Pro Phe Leu Phe Leu Ile Met His Lys Ile Thr Lys Cys Ile Leu Phe Phe Gly Arg Phe Cys Ser Pro End 410
400
TGCTTCTGCA AAGATTITTT GTAGATGAGC TGTGTGCTC AGAATTGCTA TTTCAAAATTT AGAGATGTTT TCTACATATT TCTGCTCTTC TGAACAACCTT 1370
CTGCTACCCA CTAAATATAA ACACAGAAAT AATTAGACAA TTGTCTATTA TAACATGACA ACCCTATTAA TCATTGGTC TTCTAAATG GGATCATGCC CATTAGATT 1480
TTCTTACTA TCAGTTTATT TTTATTAACAT TAACTTTAC TTTGTTTATT ATTATTTTAT ATATGCTGA GTTTTAAAT TATTGCTCAC TGCCTATTTA ATGTAGCTAA 1590
TAAAGTTATA GAAGCAGATG ATCTGTTAAT TTCCTATCTA ATAAATGCCY TTAATTGTTT TCATAATGAA GAATAAGTAG GTATCCCTCC ATGCCCCCTT ATAAATAATA 1700
TCTGGAAAAA ACATTAAACA ATAGGCAAAAT ATATGTTATG TGCATTTCTA GAAATACATA ACACATATAT ATGCTGTGAT CTTATATTCA ATTGCAAGTA TATAATAAAT 1810
AAACCTGCTT CCAACAACA AAAAAAAAAA AAAAAAAAAA AAAAA 1855

FIG. 4B.

1 3 4 1 3 1 8

5/5



FIG. 5.

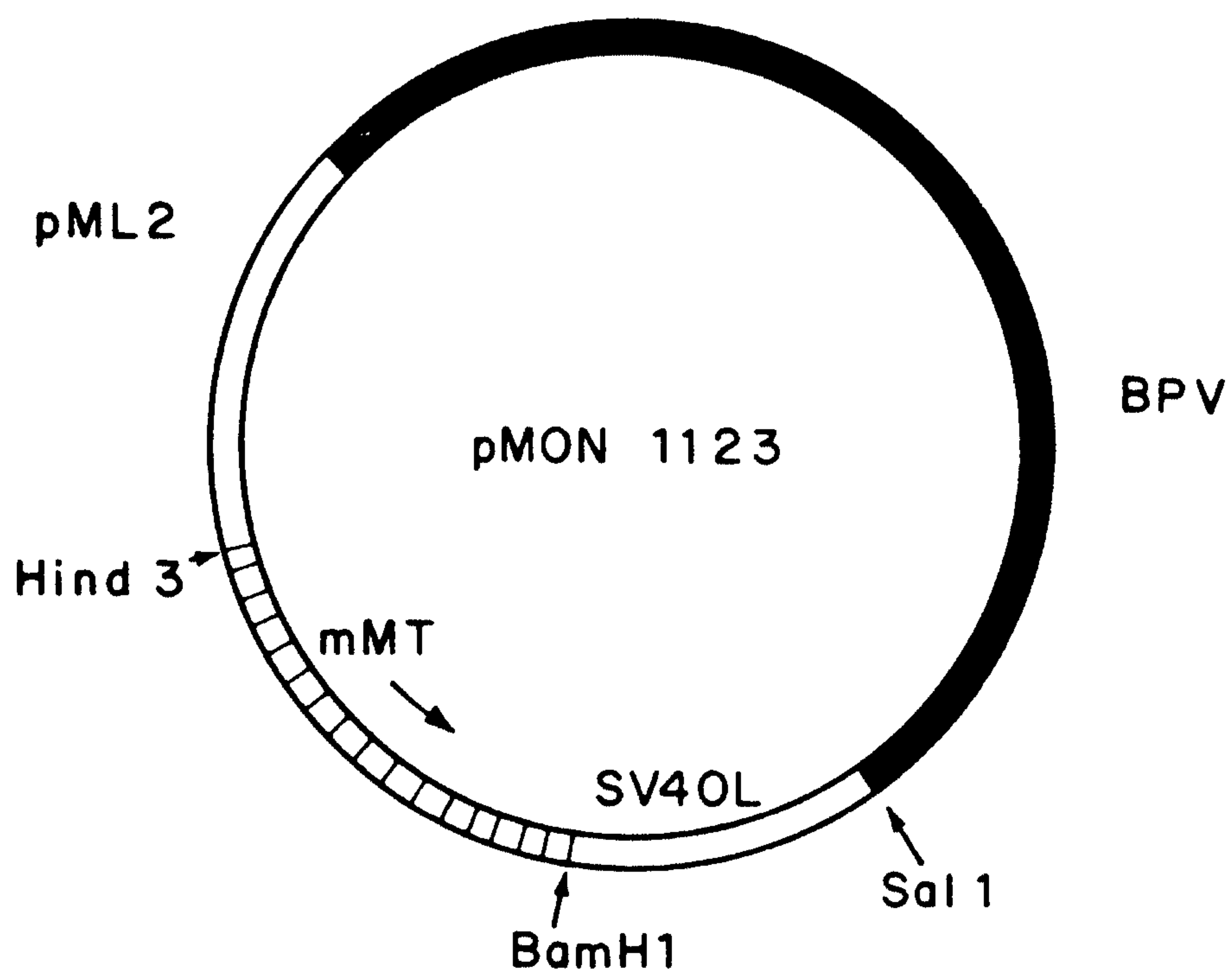


FIG. 6.