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(54) Title: HUMAN DNASE (57) Abstract The present invention discloses a human DNase polypeptide and DNA (RNA) encoding such polypeptide. The human DNase polypeptide may be produced by recombinant DNA techniques and is useful against the treatment of various diseases. In such treatment, the human DNase polypeptide is administered with a suitable pharmaceutical carrier or diluent to provide therapeutic effects against such diseases.		

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HUMAN DNASE

This invention relates to newly identified polynucleotide sequences, polypeptides encoded by such sequences, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is a human deoxyribonuclease (DNase). The invention also relates to inhibiting the action of such polypeptide.

DNase is a phosphodiesterase capable of hydrolyzing polydeoxyribonucleic acid. It acts to extensively and non-specifically degrade DNA and in this regard it is distinguished from the relatively limited and sequence-specific restriction endonucleases. The degradation activity as stated above is non-specific. However, it also degrades double stranded DNA to yield 5'-oligonucleotides. There are two types of DNase, both DNase I and II. DNase I has a pH optimum near neutrality, an obligatory requirement for divalent cations, and produces 5'-phosphate nucleotides on hydrolysis of DNA. DNase II exhibits an acid pH optimum, can be activated by divalent cations and produces 3'-phosphate nucleotides on hydrolysis of DNA. Multiple molecular forms of DNase I and II also are known.

The DNase I is mainly a digestive enzyme, however, activities similar to DNase I had been found in other tissues suggesting that it may have other functions (Laskowski, ref. 1971). For example, it has been suggested that DNase may play a role in the polymerization of actin. (Suck, et al., ref. 1981). DNase's in prokaryotic cells participate in a variety of metabolic functions, including genetic recombination, repair of DNA damage, restriction of foreign DNA and transport of DNA into cells.

DNase from various species have been purified to a varying degree. Bovine DNase A, B, C, and D was purified and completely sequenced as early as 1973 (Liao et al., J. Biol. Chem. 248:1489 [1973]). Porcine and bovine DNase have been purified and fully sequenced (Paudel et al., J. Biol. Chem. 261:16006 [1986]). Human urinary DNase was reported to have been purified to an electrophoretically homogenous state and the N-terminal amino acid observed to be leucine; no other sequence was reported (Ito et al., J. Biol. Chem. 95:1399 [1984]).

Recently, the bovine and human DNase I genes have been cloned and expressed, as shown in PCT international application number PCT/US89/05744, applied for by Genentech, Inc., which discloses human DNase isolated from the pancreas.

The Shields et al. reference described the expression cloning of part of the gene for bovine DNase I and expression of a fusion product in *E. coli* which was biologically and immunologically active (Biochem. Soc. Trans. 16:195 [1988]). The DNase product of Shields et al., however, was toxic to the host cells and could only be obtained by the use of an inducible promoter. Furthermore, great difficulty was encountered in attempts to isolate plasmid DNA from either clone, an obstacle attributed to constitutive levels of expression of DNase from the clones, so that these authors were unable to determine the sequence for the DNase-encoding nucleic acid. According to Shields et al., the inability to

recover the plasmid was the result of constitutive expression of DNase even when the promoter was repressed at low temperature. This would create a considerable obstacle since Shields et al. had only identified the clone by expression cloning, which necessarily requires that the DNase be placed under the control of a promoter of some sort.

DNase finds a number of known utilities and has been used for therapeutic purposes. Its principal therapeutic use has been to reduce the viscosity of pulmonary secretions in such diseases as pneumonia, cystic fibrosis, thereby aiding in the clearing of respiratory airways. Obstruction of airways by secretions can cause respiratory distress, and in some cases, can lead to respiratory failure and death.

In accordance with one aspect of the present invention, there is provided a novel polypeptide which is a DNase as well as analogs and derivatives thereof. The DNase of the present invention is of human origin.

In accordance with another aspect of the present invention, there is provided a polynucleotide (DNA or RNA) which encodes such polypeptide.

In accordance with still another aspect of the present invention, there is provided a procedure for producing such polypeptide by recombinant techniques.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptide or DNA sequence encoding such polypeptide for therapeutic purposes, for example, for the enzymatic alteration of the viscosity of bronchopulmonary secretions.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

FIG. 1 depicts the amino acid and DNA sequence of the human DNase of the present invention. The amino acids are represented by their standard one letter abbreviations. The protein shown is the preprocessed form of the protein.

FIG. 2 illustrates the amount of DNase released from the pelleted fraction of a bacterial expression system as opposed to the soluble fraction at different time intervals.

FIG. 3 shows the rate of success of purification of DNase in different mediums and at different pHs.

FIG. 4 demonstrates the tissue distribution of DNase through the use of a Northern blot analysis, indicating that DNase is prevalent in the lungs and heart.

FIG. 5 depicts the ability of DNase to digest double-stranded DNA.

FIG. 6 demonstrates DNase activity. DNA was labelled with ^{32}P -dCTP. DNase is then incubated with a fixed amount of labeled DNA and samples were removed and counted by liquid scintillation.

FIG. 7 is a schematic representation of the pQE-9 vector..

In accordance with one aspect of the present invention, there is provided a DNA sequence (and corresponding RNA sequence) as set forth in Figure 1 of the drawings and/or DNA (RNA) sequences encoding the same polypeptide as the sequence of Figure 1 of the drawings, as well as fragment portions, derivatives, analogs and all allelic variants of such sequences. The polypeptide of Figure 1 is the preprocessed form of the protein and has a leader sequence. The processed or mature form begins at amino acid 19.

In accordance with another aspect of the present invention, there is provided a polynucleotide which encodes the same polypeptide as the polynucleotide of the cDNA clone deposited as ATCC deposit number 75515, deposited on August

4, 1993, and/or fragments, analogs, derivatives or allelic variants of such polynucleotide.

The polynucleotide (DNA or RNA, preferably DNA) includes at least the portion coding for the polypeptide, which coding portion may be the same as that in the deposited clone or may be different than that in the deposited clone provided that it encodes for the same polypeptide or an allelic variant thereof. The coding portion preferably encodes at least the mature form of the polypeptide of the present invention.

The present invention further relates to polynucleotide sequences which hybridize to the hereinabove-described polynucleotide sequences if there is at least 50% and preferably at least 70% identity between the sequences. In another preferred embodiment the present invention relates to polynucleotide sequences which hybridize under stringent conditions to the hereinabove-described polynucleotide sequences. As herein used, the term "stringent conditions" means hybridization will occur if there is at least 95 % and preferably at least 97 % identity between the segments. Thus, the present invention includes DNA (RNA) sequences encoding allelic variant forms of the peptide encoded by the DNA of Figure 1. Thus, the present invention provides isolated DNA (RNA) encoding for a naturally occurring human polypeptide which is a DNase, as well as allelic variants thereof. The DNA (RNA) is preferably provided in a purified and isolated form.

The present invention further relates to a polypeptide which is a DNase, and which, has the structure shown in Figure 1, as well as allelic variants thereof, and analogs, fragments, derivatives and allelic variants thereof and which have the same function as the naturally occurring polypeptide.

The present invention further relates to a polypeptide encoded by the DNA contained in the clone deposited as ATCC

number 75515 on August 4, 1993, as well as analogs, fragments, derivatives and allelic variants thereof.

These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-Organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. 112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptide encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The polypeptide of the present invention is preferably provided in an isolated form, and preferably is purified.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

In a preferred embodiment, the DNase is a full length mature human DNase or an allelic or glycosylated variant thereof. The polynucleotide may also encode a preprotein which is processed and secreted from mammalian cells as the mature protein.

The polynucleotide sequence of the present invention may encode for the mature form of the polypeptide or may encode

for a leader sequence. For example, the desired DNA sequence may be fused in the same reading frame to a DNA sequence which aids in the expression and secretion of the polypeptide, for example, a leader sequence which acts as a secretory sequence for controlling transportation of the polypeptide from the cell of the host. The protein having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the protein. The polynucleotide of the present invention may also be fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention, for example, a hexa-histidine tag.

Thus, the polypeptide(s) of the present invention may be the mature form of the DNase of the present invention; or may be in the form of a preprotein or prepolyptide wherein the DNase includes a leader or secretory sequence; or may be in the form of a fusion protein wherein additional amino acids which aid in, for example, purification of the polypeptide are fused to the mature or preprotein at either the 3' or 5' end thereof.

In a preferred embodiment, the marker sequence is a hexa-histidine tag, which is supplied by the plasmid pQE-9.

As hereinabove indicated, the present invention also includes variants of the polypeptide which is encoded by the DNA of Figure 1 or and variants of the DNA contained in the deposited clone, which retains the qualitative activity of such a polypeptide which is a DNase. The variant may be a substitutional variant, or an insertion variant or a deletional variant. Such variants can be naturally occurring allelic variants such as for example, those with different glycosylation patterns or substitution at the amino acid level or deletion at the amino acid level.

Such variants may also be produced by site specific mutagenesis. The substitutional variant may be a substituted

conserved amino acid or a substituted non-conserved amino acid, and preferably a conserved amino acid.

A polynucleotide encoding a polypeptide of the present invention may be obtained from one or more libraries prepared from one of the following tissues: heart, lung, infant brain and placenta. It contains an open reading frame of 298 amino acids encoding for a preprocessed form which exhibit some homology to both human and non-human pancreatic DNase. Among the top matches are: 34% identity and 53% similarity to the human pancreatic DNase over a stretch of 282 amino acids. The coding sequence also contains regions of up to 60% identity and 75% similarity to DNase I from a pig and an overall similarity of 54%. The predicted molecular weight based on amino acid composition is 33kDa.

Host cells are transformed with the expression vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants or amplifying the DNase gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

"Transformation" means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Unless indicated otherwise, the method used herein for transformation of the host cells is the method of Graham, F. and van der Eb, A., Virology 52:456-457 (1973). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used. If prokaryotic cells or cells which contain substantial cell wall constructions are used, the preferred method of transfection is calcium treatment using calcium chloride as described by Cohen, F.N. et al., Proc. Natl. Acad. Sci. (USA), 69:2110 (1972).

"Transfection" refers to the introduction of DNA into a host cell whether or not any coding sequences are ultimately

expressed. Cells do not naturally take up DNA. Thus, a variety of technical "tricks" have been utilized to facilitate gene transfer. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, CaPO_4 and electroporation. Transformation of the host cell is the indicia of successful transfection.

The polynucleotide of the present invention may be employed for producing a polypeptide by recombinant techniques. Thus, for example, the polynucleotide sequence may be included in any one of a variety of vectors or plasmids for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA'S; yeast plasmids; vectors derived from combinations of plasmids and phage DNAs, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the *E. coli*, lac or trp, the phage lambda PL promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain a gene to provide a phenotypic trait for selection of

transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. The human DNase of the present invention as it is expressed from a bacterial expression system, e.g., *E. coli* is shown in Figure 2 and Figure 3, lane 5. As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as *E. coli*, *Salmonella typhimurium*; fungal cells, such as yeast; animal cells such as Cos-7 cells, CHO or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

The DNase of the present invention was purified from an *E. coli* host through the use of a nickel chelate resin that has a high affinity for the hexa-histidine tag.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE-9 (Qiagen), pBs, phagescript, pD10, PsiXI74, pbluescript SK, pBsKS, PNH8A, PNE16A, PNH18A, PNH46A (Stratagene); Ptrc99a, PKK223-3, PKK233-3, PDR540, PRIT5 (Pharmacia). Eukaryotic: pWLneo, PSV2CAT, POG44, PXTI, pSG (Stratagene) PSVK3, PBPV, PMSG,

PSVL (Pharmacia). Also, any other plasmids and vectors may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol acetyl transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_o and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described construct. The host cell can be a higher eukaryotic cell, such as a mammalian cell; or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, 1986).

The constructs in host cells can be used in a conventional manner to produce the gene product coded by the recombinant sequence. Alternatively, the encoded polypeptide can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook,

et al., Molecular Cloning, : A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of a DNA encoding a polypeptide of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin (bp 100 to 270), a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*,

Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector PBR322 (ATCC 37017). Such commercial vectors include, for example, PKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These PBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

DNase is recovered and purified from recombinant cell cultures by methods used theretofore with human, bovine, ovine, or porcine DNase, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation or exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography (e.g., using DNA or nucleotides on a solid support), hydroxylapatite chromatography and lectin chromatography. Moreover, reverse-phase HPLC and chromatography using anti-DNase antibodies are useful for the purification of DNase. As noted previously (Price, et al., J. Biol. Chem. 244:917 [1969]) it is preferred to have low concentrations (approximately 0.1-5mM) of calcium ion present

during purification. Other divalent cations which stabilize DNase are also utilized. DNase may be purified in the presence of a protease inhibitor such as PMSF.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more salting-outs, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

The polypeptide of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture) of a polynucleotide sequence of the present

invention. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue (at position 1).

In addition to naturally occurring allelic forms of the polypeptide, the present invention also embraces analogs and fragments thereof. Thus, for example, one or more of the amino acid residues of the polypeptide may be replaced by conserved amino acid residues.

Purified DNase of the present invention may be employed to enzymatically reduce the viscosity of mucus in the manner as described above. Based on this activity it would be of particular use for the treatment of patients with pulmonary disease who have abnormal or viscous or inspissated purulent secretions in conditions such as acute or chronic bronchial pulmonary disease (infectious pneumonia, bronchitis, or tracheobronchitis, bronchiectasis, cystic fibrosis, asthma, TB, or fungal infections), atelectasis due to tracheal or bronchial impaction and complications due to tracheostomy. For such therapies, a solution or finely divided dry preparation of human DNase is instilled in conventional fashion into the bronchi, e.g., by aerosolization of a solution of DNase.

In addition to these direct uses, DNase would have adjunctive treatment for the management of abscesses of closed spaced infections, emphysema, meningitis, peritonitis, sinusitis, otitis, periodontitis, pancreatitis, cholelithiasis, endocroditis, and septic arthritis, as well as in topical treatments in a variety of inflammatory and infected lesions, such as infected lesions of the skin and/or mucosal membranes, surgical wounds, ulcerative lesions and burns.

Human DNase finds utility in maintaining the flow in medical conduits communicating with a body cavity, including surgical drainage tubes, urinary catheters, peritoneal dialysis ports, and intratracheal oxygen catheters.

The DNase of the present invention may also improve the efficacy of antibiotics in infections. The DNase may also be useful in degrading DNA contaminants in pharmaceutical preparations. Finally, DNase may be useful in treating non-infected conditions in which there is an accumulation of cellular debris, including cellular DNA. For example, DNase will be useful after systemic administration in the treatment of pyelonephritis and tubulo-interstitial kidney disease.

The polypeptide of the present invention may also be employed in accordance with the present invention by expression of such polypeptide *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells such as lung and heart cells may be transduced with a polynucleotide (DNA or RNA) encoding the polypeptide *ex vivo*, with the transduced cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be transduced by procedures known in the art by use of a retroviral particle containing RNA encoding the polypeptide of the present invention.

Similarly, transduction of cells may be accomplished *in vivo* for expression of the polypeptide *in vivo* for example, by procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for transduction *in vivo* and expression of the polypeptide *in vivo*.

These and other methods for administering a polypeptide of the present invention by such methods should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for

transducing cells may be other than a retroviral particle, for example, an adenovirus.

For example, a polynucleotide encoding a polypeptide of the present invention is ligated into an adenoviral vector. The adenoviral vector can then be packaged in a delivery vehicle, e.g., an aerosol spray, and administered to the patient by spraying through the nasal or oral cavity into the lungs. Cells are then transfected *in vivo* with the DNA sequence encoding for the polypeptide of the present invention to produce DNase. In this manner, the DNase degrades the excessive secretions of mucous in the lungs, such as in pneumonia, and facilitates respiration.

The polypeptide of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the protein, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

For example, the human DNase of the present invention is placed into therapeutic formulations, together with required co-factors. The formulation of DNase may be liquid, lyophilized powder or the DNase may be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

When the DNase of the present invention is used as a drug, as described above, it is given, for example, in appropriate doses of at least about 10 $\mu\text{g/kg}$ body weight and in most cases it would be administered in an amount not in excess of about 8 mg/kg body weight per day, for example, in doses of about 2.5 mg/person/day, taking into account the routes of administration, symptoms, etc.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of

the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptide of the present invention may be employed in conjunction with other therapeutic compounds.

Each of the cDNA sequences identified herein or a portion thereof can be used in numerous ways as polynucleotide reagents. The sequences can be used as diagnostic probes for the presence of a specific mRNA in a particular cell type. In addition, these sequences can be used as diagnostic probes suitable for use in genetic linkage analysis (polymorphisms).

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of cDNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the cDNA is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include hybridization, prescreening with labeled flowsorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence in situ hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clone from which the EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the

affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that cDNA sequence. Ultimately, complete sequencing of genes from several individuals is required to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

The protein, its fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal, monoclonal, chimeric, single chain, Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of polyclonal antibodies.

Antibodies generated against the polypeptide corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptide into an animal or by administering the polypeptide to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies binding the whole native polypeptide. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide. Moreover, a panel of such antibodies, specific to a large number of

polypeptides, can be used to identify and differentiate such tissue.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kohler et al., 1983, Immunology Today 4:72), and the EBV--hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention.

The antibodies can be used in methods relating to the localization and activity of the protein sequences of the invention, e.g., for imaging these proteins, measuring levels thereof in appropriate physiological samples and the like.

The present invention will be further described with reference to the following examples, however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour or longer at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on an agarose gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using a 0.8- 2.0 percent polyagarose gel. (Maniatis)

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Example 1Bacterial Expression and Purification of Human DNase

The DNA sequence encoding for human DNase, pBLSKDNase (ATCC # 75515) is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end of the DNA sequence to synthesize insertion fragments. The 5' oligonucleotide primer has the sequence GACGCCGGATCCCACTACCCAACTGCA, contains a BamHI restriction enzyme site followed by 15 nucleotides of DNase coding sequence following the initiation codon; the 3' sequence 5'-G G C T G C T C T A G A C A G C G T A G T C T G G C A -GGTCGTATGGGTACTTCAGCTCCACCTCCACGGGGTAG-3' contains complementary sequences to XbaI site and is in the 3' untranslated region of the gene. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311) The plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His) and restriction enzyme cloning sites. The pQE-9 vector was digested with BamHI and XbaI and the insertion fragments were then ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. Figure 7 shows a schematic representation of this arrangement. The ligation mixture was then used to transform the *E. coli* strain available from Qiagen under the trademark m15/rep4. M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates containing both Amp and Kan. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 µg/ml) and Kan (25 µg/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250.

The cells were grown to an optical density at 600nm (O.D.⁶⁰⁰) between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalactopyranoside") was then added to a final concentration of 1mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 2-4 hours. Cells were then harvested by centrifugation. Expression of DNase was tested by solubilizing a portion of the E. coli and analyzing on a SDS polyacrylamide gel. The presence of a new protein corresponding to 33 kd following induction demonstrated expression of the DNase. (Figure 2).

Purification of protein was accomplished by utilization of the hexa-histidine sequence added to the DNase sequence by the pQE-9 vector. Following induction of cells, the E. coli pellet was solubilized in 6M guanidine HCL. The DNase was purified using a Nickel-Chelate resin column that has a high affinity for the hexa-histidine tag. (Hachuli, E. et al., Genetic Engineering, Principle and Methods, 12:87-98 Plenum Press, New York (1990)). The column was then washed, and the DNase was eluted at a pH of 5. Protein renaturation out of Guanidine HCL can be accomplished by several protocols. (Jaenicke, R. et al. Protein Structure - a practical approach IRL Press, New York (1990)). Initially, step dialysis is utilized to remove the GnHCl. Alternatively, the purified protein isolate from the Ni-chelate column can be bound to a second column over which a decreasing linear GnHCl gradient is run. The protein is allowed to renature while bound to the column and is subsequently eluted with GnHCl, pH 5.0. Finally, soluble protein is dialyzed against a storage buffer containing 140mM NaCl, 20mM NaPO₄ and 10% w/v Glyconol. The purified protein was analyzed by SDS-PAGE. (Figure 3).

Example 2

Expression of DNase in COS Cells

The expression of plasmid, soCMVDNase HA is derived from a vector SoCMVIN/Amp containing: 1) SV40 origin of replication, β globin intron, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. (Ruben, S., et. al. Molecular and Cellular Biology, 444-454 (1992). A DNA fragment encoding the entire DNase and a HA tag fused in frame to its 3' end was cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (MYPYDVPDYA) as previously described (I. Wilson, et al. Cell, 37:767 (1984). The infusion of HA tag to our target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follow:

The DNA insert was constructed by PCR on the original EST using two primers: the 5' primer GACGCCGGATCCCACTACCCAACTGCA contains a BamHI site followed by 15 nucleotides of DNase coding sequence starting from the initiation codon; the 3' sequence GGCTGCTCTAGACAGCGTAGTCTGGCAGGTCGTATGGG-TACTTCAGCTCCACCTCCACGGGGTAG contains complementary sequences to the XbaI site, translation stop codon, HA tag and the 23 nucleotides of the DNase coding sequence (not including the stop codon) that correspond to pancreatic DNase terminal residues. Therefore, the PCR product contains a BamHI site, DNase coding sequence, HA tag fused in frame, followed by a translation termination stop codon next to the HA tag, and a XbaI site. The PCR amplified DNA fragment and the vector, SoCMVIN/amp, were digested with BamHI and XbaI restriction enzyme and ligated. The ligation mixture was transformed into E. coli strain available under the trademark SURE. The transformed culture was plated on ampicillin media plates and

resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant DNase, COS cells were transfected with the expression vector by DEAE-DEXTRAN method. (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, 1989.) The expression of the DNase-HA protein was detected by radiolabelling and immunoprecipitation method. Cells were labelled for 8 hours with ³⁵S-cysteine two days post transfection. (E. Harlow, D. Lane, Antibodies: A Laboratory manual, Cold Spring Harbor Laboratory Press, (1988)). Culture media were then collected and cells were lysed with detergent. (RIPA buffer (150mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH7.5). (I. Wilson et al. Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.

Example 3

Tissue Distribution of DNase

To demonstrate tissue distribution of DNase, Northern blot analysis was performed. Total RNA was prepared from various human tissues and electrophoresed on denaturing agarose gels. The RNA was blotted onto nylon membranes and probed with a DNase cDNA probe corresponding to the EcoRI-Xho fragment of pBLSKDNase prepared by random priming. Exposure of the blot to film revealed that DNase is most abundant in tissue from the heart and lungs with some expression in the spleen. (Figure 4). Total cellular RNA samples were isolated with RNazol™ B system (Biotechx Laboratories, Inc., 6023 South Loop East, Houston, TX 77033.) About 10ug of total RNA isolated from each human tissue specified was separated on 1% agarose gel and blotted onto a nylon filter (Sambrook, Fritsch, and Maniatis, Molecular Cloning, Cold Spring Harbor

Press, (1989)). The labeling reaction was done according to the Stratagen Prime-It kit with 50 ng of the Eco RI-XhoI fragment of pBL s1e DNase DNA fragment. The labeled DNA was purified with a Seclect-G-50 column. (5 Prime -- 3 Prime, Inc. 5603 Arapahoe Road, Boulder, CO 80303). The filter was then hybridized with radioactive labeled full length thrombin inhibitor gene at 1,000,000 cpm/ml in 0.5 M NaPO₄ and 7% SDS overnight at 65°C. After wash twice at room temperature and twice at 60°C with 0.5 x SSC, 0.1% SDS, the filters were then exposed at -70°C overnight with intensifying screen. (Figure 4).

Example 4Assays for DNase Activity

Several assays are available to measure DNase Activity. The first assay measures the hydrolysis of ^{32}P -labeled DNA.

Degradation of ^{32}P -DNA was prepared using a Random Prime Labeling Kit (Stratagene's Prime It II) using ^{32}P -dCTP, non-radioactive dGTP, dTTP, dATP and Exo (-) Klenow Enzyme. 1 ul of template (25 ng), 10ul of random oligonucleotide primers and 23 ul of water were mixed and incubated at 100°C for 5 minutes. 10 ul of 5X dCTP primer buffer, 5 ul of ^{32}P -dCTP (3.000 Ci/mMole) and 1 ul Exo (-) Klenow Enzyme (5U/ul) were added to the reaction mixture and incubated at 37°C for 15 minutes. The free nucleotides were separated from the radiolabeled DNA by centrifugation through a Sephadex G-50 column.

DNase activity was then measured by incubating 5×10^5 cpm ^{32}P -DNA and 20 ul of protein in DNase buffer (40mM TRIS-HCl pH 7.6, 50mM NaCl, 0.1mM DTT, 6mM MgCl_2 , 1mM CaCl_2) in a total volume of 100ul. 20ul were removed for time point 0 and for each subsequent time point.

TCA precipitable counts were used to determine % activity. (Figure 6).

To the 20 ul of the DNase assay reaction mixture, 20ul of 10% TCA was added and mixed. 20ul was spotted onto a glass filter disk (unincorporated counts). To the remaining 20ul, an additional 200 ul of 10% TCA was added and then applied to a prewet glass filter disk on a vacuum manifold and the vacuum applied. The disks were washed twice with 3 ml 10% TCA then twice with 3 ml 100% Ethanol and the filter allowed to dry. The filter disks were then place into a liquid scintillation vial containing 3 ml of liquid scintillation cocktail and ^{32}P counts were measured in a Liquid scintillation counter. Percent incorporation = Incorporated Counts/Unincorporated counts.

A second assay takes advantage of the ability of DNase to nick and digest double-stranded DNA. Supercoiled plasmid DNA is incubated with DNase and samples are removed at time points following addition of the DNase. The samples are electrophoresed on agarose gels and the conversion from supercoiled to relaxed DNA is observed following addition of the DNase. (Figure 5).

A rapid plate DNase assay can also be used in which test agar contains both DNA and methyl green. Purified protein or supernatants from transfected cells are spotted on the plates along with a standard amount of bovine DNase. DNase activity is measured by visualizing the size of the cleared zone on the plate.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

P:\op\PATAP090

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: RUBEN, ET AL.
- (ii) TITLE OF INVENTION: Human DNase
- (iii) NUMBER OF SEQUENCES: 2
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN,
CECCHI, STEWART & OLSTEIN
 - (B) STREET: 6 BECKER FARM ROAD
 - (C) CITY: ROSELAND
 - (D) STATE: NEW JERSEY
 - (E) COUNTRY: USA
 - (F) ZIP: 07068
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: 3.5 INCH DISKETTE
 - (B) COMPUTER: IBM PS/2
 - (C) OPERATING SYSTEM: MS-DOS
 - (D) SOFTWARE: WORD PERFECT 5.1
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/172,414
 - (B) FILING DATE: 22 DECEMBER 1993
 - (C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: FERRARO, GREGORY D.
 - (B) REGISTRATION NUMBER: 36,134
 - (C) REFERENCE/DOCKET NUMBER: 325800-54

(viii) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 201-994-1700

(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1055 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGCCATGCAC	TACCCAACTG	CACTCCTCTT	CCTCATCCTG	GCCAATGGGA	CCCAGACCTT	60
TCGCATCTGC	GCCTTCAATG	CCCAGCGGCT	GACACTGCCC	AAGGTGGCCA	GGGAGCAGGT	120
GATGGACACC	TTAGTTCGGA	TACTGGCTCG	CTGTGACATC	ATGGTGCTGC	AGGAGGTGGT	180
GGACTCTTCC	GGCAGCGCCA	TCCCGCTCCT	GCTTCGAGAA	CTCAATCGAT	TTGATGGCTC	240
TGGGCCCTAC	AGCACCTGA	GCAGCCCCCA	GCTGGGGCGC	AGCACCTACA	TGGAGACGTA	300
TGTGTACTTC	TATCGGTCAC	ACAAAACACA	GGTCCTGAGT	TCCTACGTGT	ACAACGATGA	360
GGATGACGTC	TTTGCCCGGG	AGCCATTTGT	GGCCCAGTTC	TCTTTGCCCA	GCAATGTCCT	420
TCCCAGCCTG	GTGTTGGTCC	CGGTGCACAC	CACTCCTAAG	GCCGTAGAGA	AGGAGCTGAA	480
CGCCCTCTAC	GATGTGTTTC	TGGAGGTCTC	CCAGCACTGG	CAGAGCAAGG	ACGTGATCCT	540
GCTTGGGGAC	TTCAATGCTG	ACTGCGCTTC	ACTGACCAAA	AAGCGCCTGG	ACAAGCTGGS	600
GCTGCGGACT	GAGCCAGGCT	TCCACTGGGT	GATTGCCGAT	GGGGAGGACA	CCACAGTGCG	660
GGCCAGCACC	CACTGCACCT	ATGACCGCGT	CGTGCTGCAC	GGGGAGCGCT	GCCGGAGTCT	720
GCTGCACACT	GCGGCTGCCT	TTGACTTCCC	CACGAGCTTC	CAGCTCACCG	AGGAGGAGGC	780
CCTCAACATC	AGTGACCACT	ACCCCGTGGA	GGTGGAGCTG	AAGCTGAGCC	AGGCGCACAG	840
CGTCCAGCCT	CTCAGCCTCA	CTGTTCTGTT	GCTGCTATCA	CTCCTGTCCC	CTCAGCTGTG	900
CCCTGCTGCC	TGAGCGTCCC	CCTACCCCCC	CAGGGCCTGC	TGCCTTTTGG	GACTTAAACC	960
CCAGCCTCCC	CCGTCCATCC	AGCCCTGGGG	CTGGGGGGCT	TCAACTATAG	TTGCCCTGTG	1020
ACTGTAGTCC	ACCCCTGCCT	GCCTTGTTTG	ATTG			1055

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 346 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala	Met	His	Tyr	Pro	Thr	Ala	Leu	Leu	Phe	Leu	Ile	Leu	Ala	Asn	-15	-10	-5
Gly	Thr	Gln	Thr	Phe	Arg	Ile	Cys	Ala	Phe	Asn	Ala	Gln	Arg	Leu	1	5	10
Thr	Leu	Pro	Lys	Val	Ala	Arg	Glu	Gln	Val	Met	Asp	Thr	Leu	Val	15	20	25
Arg	Ile	Leu	Ala	Arg	Cys	Asp	Ile	Met	Val	Leu	Gln	Glu	Val	Val	30	35	40
Asp	Ser	Ser	Gly	Ser	Ala	Ile	Pro	Leu	Leu	Leu	Arg	Glu	Leu	Asn	45	50	55
Arg	Phe	Asp	Gly	Ser	Gly	Pro	Tyr	Ser	Thr	Leu	Ser	Ser	Pro	Gln	60	65	70
Leu	Gly	Arg	Ser	Thr	Tyr	Met	Glu	Thr	Tyr	Val	Tyr	Phe	Tyr	Arg	75	80	85
Ser	His	Lys	Thr	Gln	Val	Leu	Ser	Ser	Tyr	Val	Tyr	Asn	Asp	Glu	90	95	100
Asp	Asp	Val	Phe	Ala	Arg	Glu	Pro	Phe	Val	Ala	Gln	Phe	Ser	Leu	105	110	115
Pro	Ser	Asn	Val	Leu	Pro	Ser	Leu	Val	Leu	Val	Pro	Leu	His	Thr	120	125	130
Thr	Pro	Lys	Ala	Val	Glu	Lys	Glu	Leu	Asn	Ala	Leu	Tyr	Asp	Val	135	140	145
Phe	Leu	Glu	Val	Ser	Gln	His	Trp	Gln	Ser	Lys	Asp	Val	Ile	Leu	150	155	160
Leu	Gly	Asp	Phe	Asn	Ala	Asp	Cys	Ala	Ser	Leu	Thr	Lys	Lys	Arg	165	170	175
Leu	Asp	Lys	Leu	Glu	Leu	Arg	Thr	Glu	Pro	Gly	Phe	His	Trp	Tyr	180	185	190

Ile Ala Asp Gly Glu Asp Thr Thr Val Arg Ala Ser Thr His Cys	195	200	205
Thr Tyr Asp Arg Val Val Leu His Gly Glu Arg Cys Arg Ser Leu	210	215	220
Leu His Thr Ala Ala Ala Phe Asp Phe Pro Thr Ser Phe Gln Leu	225	230	235
Thr Glu Glu Glu Ala Leu Asn Ile Ser Asp His Tyr Pro Val Glu	235	240	245
Val Glu Leu Lys Leu Ser Gln Ala His Ser Val Gln Pro Leu Ser	250	255	260
Leu Thr Val Leu Leu Leu Leu Ser Leu Leu Ser Pro Gln Leu Cys	265	270	275
Pro Ala Ala Xaa Ala Ser Pro Tyr Pro Pro Arg Ala Cys Cys Leu	280	285	290
Leu Gly Leu Lys Pro Gln Pro Pro Pro Ser Ile Gln Pro Trp Gly	295	300	305
Trp Gly Ala Ser Thr Ile Val Ala Leu Xaa Leu Xaa Ser Thr Pro	310	315	320
Ala Cys Leu Val Xaa Phe	325		

WHAT IS CLAIMED IS:

1. An isolated DNA sequence comprising DNA encoding at least the same mature polypeptide as the DNA sequence of Figure 1 or any allelic variants thereof.
2. The isolated DNA of claim 1 wherein said DNA comprises the DNA of Figure 1 encoding for at least the mature polypeptide of Figure 1 and allelic variants thereof.
3. An isolated RNA sequence comprising RNA and allelic variants thereof corresponding to any of the DNA sequences of claim 1.
4. An isolated DNA sequence comprising a DNA sequence encoding at least a mature polypeptide identical to the mature polypeptide encoded by the DNA sequence contained in ATCC Deposit No. 75515 and allelic variants thereof.
5. The isolated DNA of claim 4 wherein said DNA comprises at least the DNA contained in ATCC Deposit No. 75515 which encodes the mature polypeptide.
6. An isolated RNA sequence comprising RNA corresponding to the DNA sequence of claim 4.
7. An expression vehicle comprising the DNA sequence of claim 1.
8. An expression vehicle comprising the DNA sequence of claim 4.
9. A polypeptide comprising an amino acid sequence encoded by by the DNA sequence of claim 1 or an active fragment, derivative or functional analogue thereof.
10. A polypeptide comprising an amino acid sequence encoded by the DNA sequence of claim 4 or an active fragment, derivative or functional analogue thereof.
11. A polypeptide comprising an amino acid sequence encoded by the DNA sequence of claim 2 or an active fragment, derivative or functional analogue thereof.
12. A polypeptide comprising an amino acid sequence encoded by the DNA sequence of claim 5 or an active fragment, derivative or functional analogue thereof.

13. A process for producing a human DNase polypeptide comprising: expressing a polypeptide by use of the DNA of claim 1.
14. An antibody against the polypeptide of claim 9.
15. An antibody against the polypeptide of claim 10.
16. Cells engineered with DNA of claim 1.
17. A process for producing cells capable of expressing a polypeptide comprising: genetically engineering cells with DNA of claim 1.
18. A method for the treatment of a patient having need of a DNase comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 9.
19. A method for the treatment of a patient to inhibit a DNase comprising: administering to the patient a therapeutically effective amount of the antibody of claim 14.
20. An isolated DNA sequence hybridizable to the DNA sequence of claim 1.

FIG. 1A

AGCCAIGCACTACCCAACTGCACCTCCTCTTCTCCTCATCCTGGCCAATGGACCCAGACCTTTCGCATCTGC
70
A M H Y P T A L L F L I L A N G T Q T F R I C
GCCTTCAATGCCAGCGGCTGACACTGCCCAAGGTGCCAGGAGCAGGTGATGGACACCTTAGTTCGGA
140
A F N A Q R L T L P K V A R E Q V M D T L V R
TACTGGCTCGCTGTGACATCATGGTGTGCAGGAGGTGGTGGACTCTTCCGGCAGCGCATCCCGCTCCT
210
I L A R C D I M V L Q E V V D S S G S A I P L L
GCTTCGAGAACTCAATCGATTGATGGCTCTGGGGCCCTACAGCACCCCTGAGCAGCCCCAGCTGGGGCGC
280
L R E L N R F D G S G P Y S T L S S P Q L G R
AGCACCTACATGGAGACGTATGIGTACTTCTATCGGTGCACACAAACACAGGTCTCTGAGTTCCTACGTGT
350
S T Y M E T Y V Y F Y R S H K T Q V L S S Y V

MATCH WITH FIG. 1B

FIG. 1B

MATCH WITH FIG. 1A

A C A A C G A T G A G G A T G A C G T C T T T G C C C G G G A G C C A T T T G T G G C C C A G T T C T C T T T G C C C C A G C A A T G T C C T
 420

Y N D E D D V F A R E P F V A Q F S L P S N V L

T C C A G C C T G G T G T T G G T C C C G C T G C A C A C C A C T C C T A A G G C C G T A G A G A G G A G C T G A A C G C C C C T C T A C
 490

P S L V L V P L H T T P K A V E K E L N A L Y

G A T G T G T T C T G G A G G T C T C C C A G C A C T G G C A G A G C A G G A C G T G A T C T G C T T G G G A C T T C A A T G C T G
 560

D V F L E V S Q H W Q S K D V I L L G D F N A

A C T G C G C T T C A C T G A C C A A A A G C C C T G G A C A A G C T G G A G C T G C G G A C T G A G C C A G G C T T C C A C T G G G T
 630

D C A S L T K K R L D K L E L R T E P G F H W V

G A T T G C C G A T G G G A G A C A C C A G T G C G G G C C A G C A C C C A C T G C A C C T A T G A C C G C G T C G T G C T G C A C
 700

I A D G E D T T V R A S T H C T Y D R V V L H

MATCH WITH FIG. 1C

MATCH WITH FIG. 1B

GGGAGCGCTGCCGGAGTCTGCTGCACACTGGGCTGCCTTIGACITCCCCACGAGCTTCCAGTCAACCG 770
 G E R C R S L L H T A A A F D F P T S F Q L T
 AGGAGGAGGCCCTCAACATCAGTGACCACCTACCCCTGGAGTGGAGCTGAAGCTGAGCCAGGCGCACAG 840
 E E A L N I S D H Y P V E V E L K L S Q A H S
 CGTCCAGCCTCTCAGCCTCACTGTCTCTGCTGCTATCACTCCTGTCCCCTCAGCTGTGCCCTGCTGCC 910
 V Q P L S L T V L L L L S L L S P Q L C P A A
 TGAGCGTCCCCCTACCCCCCAGGGCCTGCTGCCCTTTGGACTTAAACCCAGCCTCCCCCGTCCATCC 980
 A S P Y P P R A C C L L G L K P Q P P S I
 AGCCCTGGGCTGGGGGCTTCAACTATAGTGGCCTGTGACTGTAGTCCACCCCTGCCCTTGTITG 1050
 Q P W G W G A S T I V A L L S T P A C L V

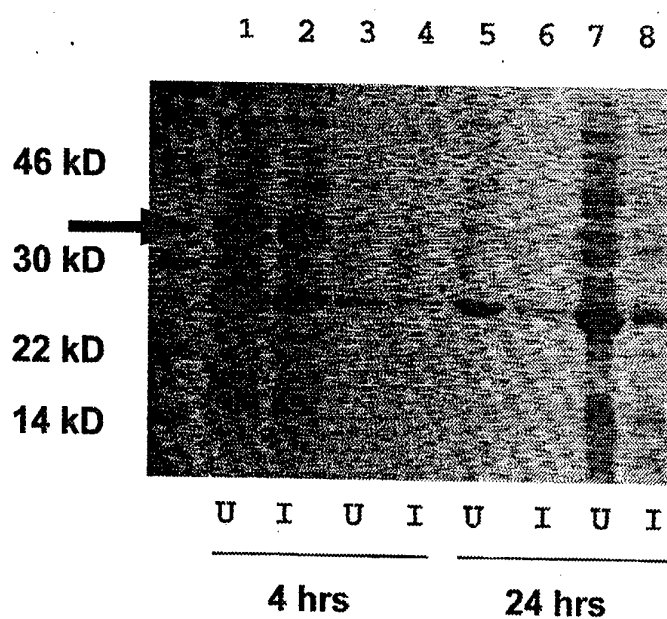
ATTG

1055

F

FIG. 1C

FIG. 2

INDUCTION OF PUTATIVE
DNase

Lanes 1,2,5,6 pelleted fraction
Lanes 3,4,7,8 soluble fraction

FIG. 3

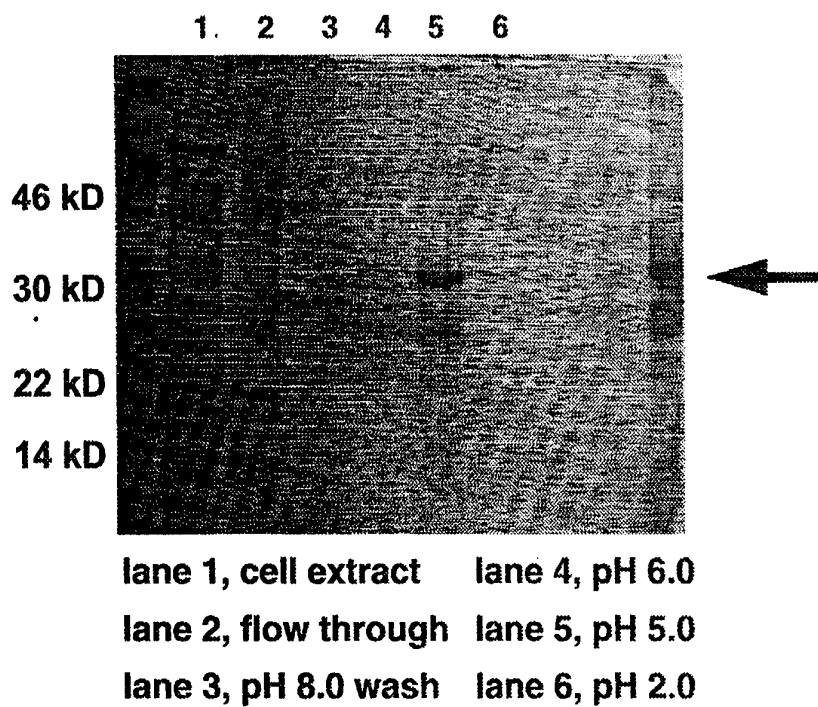
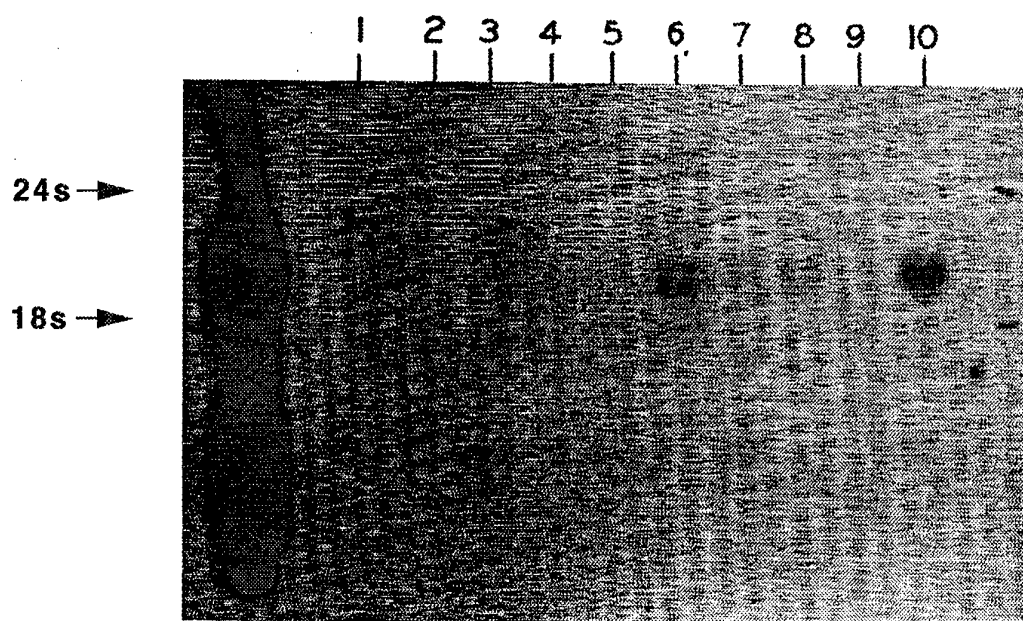
PURIFICATION OF
DNase

FIG. 4

NORTHERN ANALYSIS OF
PUTATIVE DNase
EXPRESSION

Lane 1 ovary

Lane 2 testes

Lane 3 gall bladder

Lane 4 kidney

Lane 5 liver

Lane 6 lung

Lane 7 spleen

Lane 8 prostate

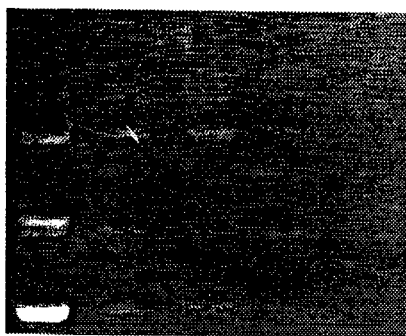
Lane 9 hippocampus

Lane 10 heart

FIG. 5

DEGRADATION OF SUPERCOILED DNA

ul DNase bl 0 5 10 20



bl-bluescript control

FIG. 6

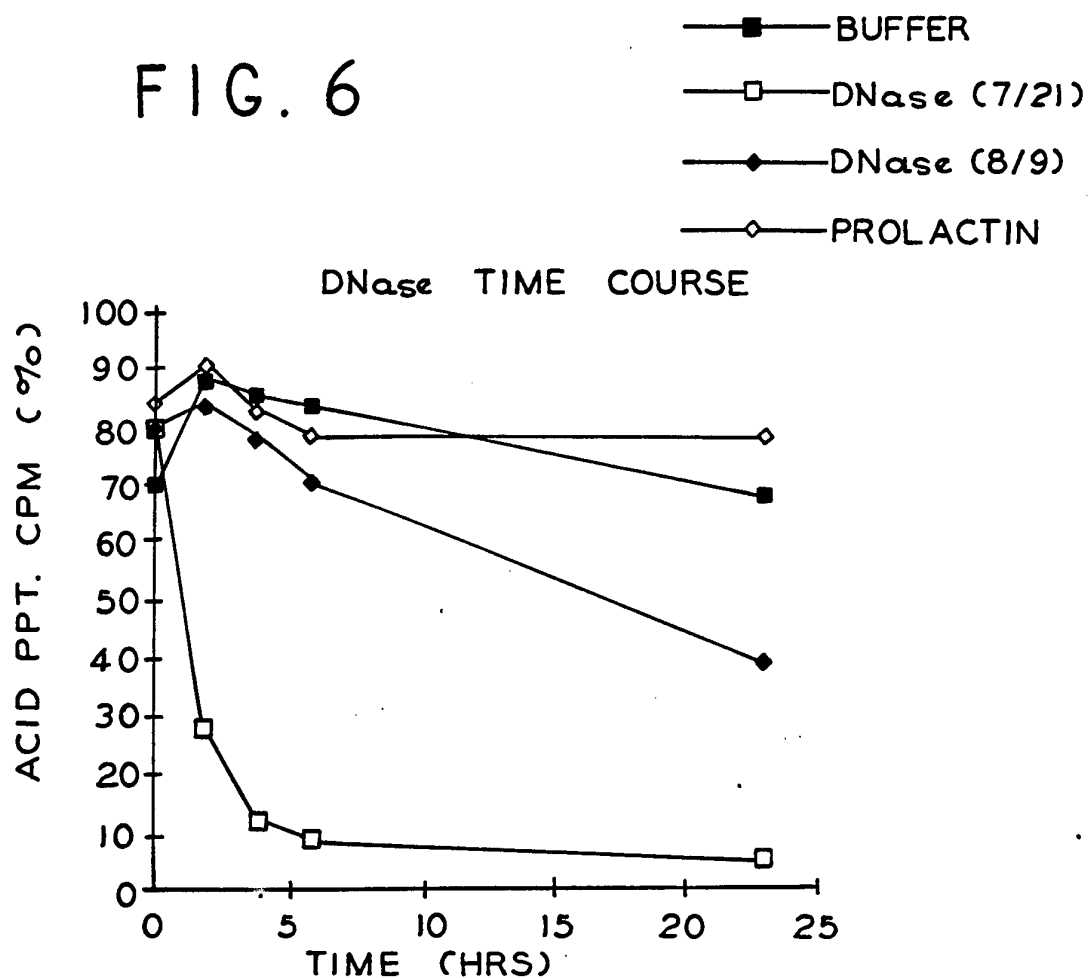
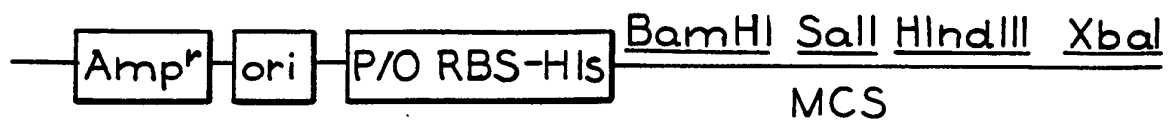


FIG. 7



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04954**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :A61K 37/54; C07K 15/28; C12N 9/22, 15/55, 15/85

US CL :536/23.2; 435/69.1, 199, 240.2, 320.1; 530/388.26, 424/94.61

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U S : 536/23.2; 435/69.1, 199, 240.2, 320.1; 530/388.26, 424/94.61

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, CAS ONLINE, WORLD PATENTS INDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US, A, 5,279,823 (FRENZ ET AL) 18 JANUARY 1994, cols. 25-26.	1-21
Y	WO, A, 90/07572 (SHAK) 12 JULY 1990, see entire document.	1-21
Y	Journal of Biochemistry, Volume 95, No. 5, issued 1984, K. Ito et al, "Human Urine DNase I: Immunological Identity with Human Pancreatic DNase I, and Enzymatic and Proteochemical Properties of the Enzyme", pages 1399-1406, see entire document.	1-21
Y	Journal of Biochemistry, Volume 82, No. 6, issued 1977, A. Funakoshi et al, "Purification and Properties of Human Pancreatic Deoxyribonuclease I", pages 1771-1777, see entire document.	1-21



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

18 JULY 1994

Date of mailing of the international search report

JUL 28 1994

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/04954

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochemical Society Transactions, Volume 16, issued 1988, D. Shields et al, "Cloning of Part of the Gene for Bovine Deoxyribonuclease I", pages 195-196, see entire document.	1-21