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(54) **GOODPASTURE ANTIGEN BINDING PROTEIN**

DAS GOODPASTURE-ANTIGEN BINDENDE PROTEIN

PROTEINE DE LIAISON A L'ANTIGENE DU SYNDROME DE GOODPASTURE

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•The file contains technical information submitted after the application was filed and not included in this specification

Description**Cross Reference**

5 [0001] This application claims priority to U.S. Provisional Patent Application Serial No. 60/121,483, filed February 24, 1999.

Statement of Government Rights

10 [0002] This work was supported in part by Grants SAL91/0513, SAF94/1051 and SAF97/0065 from the Plan Nacional I+D of the Comisión Interministerial de Ciencia Tecnología (CICYT, Spain), Grant 93/0343 from Fondo de Investigaciones Sanitarias (FISs, Spain) and Grants GV-3166/95, GV-C-VS-21-118-96 from la Direcció General d'Ensenyaments Universitaris i Investigació (Comunitat Valenciana, Spain); therefore the State of Spain may have rights in the invention.

Field of the Invention

15 [0003] The invention relates to the fields of protein kinases, automimmune disease, apoptosis, and cancer.

Background of the Invention

20 [0004] Goodpasture (GP) disease is an autoimmune disorder described only in humans. In GP patients, autoantibodies against the non-collagenous C-terminal domain (NC1) of the type IV collagen $\alpha 3$ chain ("Goodpasture antigen") cause a rapidly progressive glomerulonephritis and often lung hemorrhage, the two cardinal clinical manifestations of the GP syndrome (see 1 for review. The reference numbers in this section correspond to reference list of Example 1).

25 [0005] The idea that common pathogenic events exist at least for some autoimmune disorders is suggested by the significant number of patients displaying more than one autoimmune disease, and also by the strong and common linkage that some of these diseases show to specific MHC haplotypes (31, 32). The experimental observation that the autoantigen is the leading moiety in autoimmunity and that a limited number of self-components are autoantigenic (31), suggest that these self-components share biological features with important consequences in self/non-self recognition
30 by the immune system. One possibility is that triggering events, by altering different but specific self-components, would result in abnormal antigen processing. In certain individuals expressing a particular MHC specificity, the abnormal peptides could be recognized by non-tolerized T cells and trigger an immune response (1).

[0006] We have previously explored the GP antigen to identify biological features of relevance in autoimmune patho-
35 genesis. Since the NC1 domain is a highly conserved domain among species and between the different type IV collagen α chains ($\alpha 1$ - $\alpha 6$) (2), the exclusive involvement of the human $\alpha 3$ (IV)NC1 in a natural autoimmune response suggests that this domain has structural and/or biological peculiarities of pathogenic relevance. Consistent with this, the N-terminus of the human antigen is highly divergent, and it contains a unique five-residue motif (KRGDS⁹) that conforms to a functional phosphorylation site for type A protein kinases (3, 4). Furthermore, the human $\alpha 3$ gene, but not the other related human or homologous genes from other species, is alternatively spliced and generates multiple transcripts also
40 containing the phosphorylatable N-terminal region (5-7). Recent studies indicate that the phosphorylation of the N-terminus of the GP antigen by cAMP-dependent protein kinase is up regulated by the presence of the alternative products (see Example 3 below). Specific serine phosphorylation and pre-mRNA alternative splicing are also associated with the biology of other autoantigens including the acetylcholine receptor and myelin basic protein (MBP) (4). The latter is suspected to be the major antigen in multiple sclerosis (MS), another exclusively human autoimmune disease in which
45 the immune system targets the white matter of the central nervous system. GP disease and MS are human disorders that display a strong association with the same HLA class II haplotype (HLA DRB1*1501)(32, 33). This, along with the recent report of death by GP disease of an MS patient carrying this HLA specificity (34), supports the existence of common pathogenic events in these human disorders.

[0007] Thus, specific serine/threonine phosphorylation may be a major biological difference between the human GP
50 antigen, the GP antigens of other species, and the homologous domains from the other human α (IV) chains, and might be important in pathogenesis (1, 4).

[0008] Therefore, the identification and isolation of the specific serine/threonine kinase that phosphorylates the N-ter-
55 minal region of the human GP antigen would be very advantageous for the diagnosis and treatment of GP syndrome, and possibly for other autoimmune disorders. Reference 42 describes polynucleotides and polypeptides of GP.

Summary of the Invention

[0009] The present invention fulfills the need in the art for the identification and isolation of a serine/threonine kinase

that specifically binds to and phosphorylates the unique N-terminal region of the human GP antigen. In one aspect, the present invention provides nucleic acid sequences encoding various forms of the Goodpasture antigen binding protein (GPBP), as well as recombinant expression vectors operatively linked to the GPBP-encoding sequences.

[0010] In another aspect, the present invention provides host cells that have been transfected with the recombinant expression vectors. In a further aspect, the present invention provides substantially purified GPBP and antibodies that selectively bind to GPBP. In still further aspect, the invention provides methods for detecting the presence of GPBP or nucleic acids encoding GPBP.

[0011] In a further aspect, the present invention provides methods for detecting the presence of an autoimmune condition or apoptosis, which comprises detecting an increase in the expression of GPBP in a tissue compared to a control tissue.

[0012] In another aspect, the present invention provides methods and pharmaceutical compositions for treating an autoimmune disorder, apoptosis, or a tumor, comprising modifying the expression or activity of GPBP in a patient in need thereof.

Brief Description of the Figures

[0013]

Figure 1. **Nucleotide and derived amino acid sequences of n4'**. The denoted structural features are from 5' to 3' end: the cDNA present in the original clone (HeLa1) (dotted box), which contains the PH homology domain (in black) and the Ser-Xaa-Yaa repeat (in gray); the heptad repeat of the predictable coiled-coil structure (open box) containing the bipartite nuclear localization signal (in gray); and a serine-rich domain (filled gray box). The asterisks denote the positions of in frame stop codons.

Figure 2. **Distribution of GPBP in human tissues (Northern blot) and in eukaryotic species (Southern blot).**

A random primed ³²P-labeled HeLa1 cDNA probe was used to identify homologous messages in a Northern blot of poly(A⁺)RNA from the indicated human tissues (panel A) or in a Southern blot of genomic DNA from the indicated eukaryotic species (panel B). Northern hybridization was performed under highly stringent conditions to detect perfect matching messages and at low stringency in the Southern to allow the detection of messages with mismatches. No appreciable differences in the quality and amount of each individual poly A⁺ RNA was observed by denaturing gel electrophoresis or when probing a representative blot from the same lot with human β-actin cDNA. The numbers denote the position and the sizes in kb of the RNA or DNA markers used.

Figure 3. **Experimental determination of the translation start site.** In (A), the two cDNAs present in pc-n4' and pc-FLAG-n4' plasmids used for transient expression are represented as black lines. The relative position of the corresponding predicted (n4') or engineered (FLAG-n4') translation start site is indicated (Met). In (B), the extracts from control (-), pc-n4'(n4') or pc-FLAG-n4' (FLAG-n4') transfected 293 cells were subjected to SDS-PAGE under reducing conditions in 10% gels. The separated proteins were transferred to a PVDF membrane (Millipore) and blotted with the indicated antibodies. The numbers and bars indicate the molecular mass in kDa and the relative positions of the molecular weight markers, respectively.

Figure 4. **Characterization of rGPBP from yeast and 293 cells.** In (A), 1 μg (lane 1) or 100 ng (lanes 2 and 3) of yeast rGPBP were analyzed by reducing SDS-PAGE in a 10% gel. The separated proteins were stained with Coomassie blue (lane 1) or transferred and blotted with anti-FLAG antibodies (lane 2) or Mab14, a monoclonal antibody against GPBP (lane 3). In (B), the cell extracts from GPBP-expressing yeast were analyzed as in A and blotted with anti-FLAG (lane 1), anti-PSer (lane 2), anti-PThr (lane 3) or anti-PTyr (lane 4) monoclonal antibodies respectively. In (C), 200 ng of either yeast rGPBP (lane 1), dephosphorylated yeast rGPBP (lane 2) or 293 cells-derived rGPBP (lane 3) were analyzed as in B with the indicated antibodies. In (D), similar amounts of H₃³²PO₄-labeled non-transfected (lanes 1), stable pc-n4' transfected (lanes 2) or transient pc-FLAG-n4' expressing (lanes 3) 293 cells were lysed, precipitated with the indicated antibodies and analyzed by SDS-PAGE and autoradiography. The molecular weight markers are represented with numbers and bars as in Figure 3. The arrows indicate the position of the rGPBP.

Figure 5. **Recombinant GPBP contains a serine/threonine kinase that specifically phosphorylates the N-terminal region of the human GP antigen.** To assess phosphorylation, approximately 200 ng of yeast rGPBP was incubated with [γ]³²P-ATP in the absence (A and B) or presence of GP antigen-derived material (C). In (A), the mixture was subjected to reducing SDS-PAGE (10% gel) and autoradiographed. In (B), the mixture was subjected to ³²P-phosphoamino acid analysis by two-dimensional thin-layer chromatography. The dotted circles indicate the position of ninhydrin stained phosphoamino acids. In (C), the phosphorylation mixtures of the indicated GP-derived material were analyzed by SDS-PAGE (15% gel) and autoradiography (GPpep1 and GPpep1Ala⁹) or immunoprecipitated with Mab 17, a monoclonal antibody that specifically recognize GP antigen from human and bovine origin, and analyzed by SDS-PAGE (12.5%) and autoradiography (rGP, GP). The relative positions of rGPBP (A), rGP

antigen and the native human and bovine GP antigens (C) are indicated by arrows. The numbers and bars refer to molecular weight markers as in previous Figures.

Figure 6. **In-blot renaturation of the serine/threonine kinase present in rGPBP.** Five micrograms of rGPBP from yeast were in-blot renatured. The recombinant material was specifically identified by anti-FLAG antibodies (lane 1) and the *in situ* ³²P-incorporation detected by autoradiography (lane 2). The numbers and bars refer to molecular weight markers as in previous Figures. The arrow indicates the position of the 89 kDa rGPBP polypeptide.

Figure 7. **Immunological localization of GPBP in human tissues.** Rabbit serum against the N-terminal region of GPBP (1:50) was used to localize GPBP in human tissues. The tissues shown are kidney (A) glomerulus (B), lung (C), alveolus (D), liver (E), brain (F), testis (G), adrenal gland (H), pancreas (I) and prostate (J). Similar results were obtained using anti-GPBP affinity-purified antibodies or a pool of culture medium from seven different GPBP-specific monoclonal antibodies (anti-GPBP Mabs 3, 4, 5, 6, 8, 10 and 14). Rabbit pre-immune serum did not stain any tissue structure in parallel control studies. Magnification was 40X except in B and D where it was 100X.

Figure 8. **GPBP Δ 26 is a splicing variant of GPBP.** (A) Total RNA from normal skeletal muscle was retrotranscribed using primer 53c and subsequently subjected to PCR with primers 11m-53c (lane 2) or 15m-62c (lane 4). Control amplifications of a plasmid containing GPBP cDNA using the same pairs of primers are shown in lanes 1 and 3. Numbers on the *left* and *right* refer to molecular weight in base pairs. The region missing in the normal muscle transcript was identified and its nucleotide sequence (*lower case*) and deduced amino acid sequence (*upper case*) are shown in (B). A clone of genomic DNA comprising the cDNA region of interest was sequenced and its structure is drawn in (C), showing the location and relative sizes of the 78-bp exon spliced out in GPBP Δ 26 (*black box*), adjacent exons (*gray boxes*), and introns (*lines*). The size of both intron and exons is given and the nucleotide sequence of intron-exon boundaries is presented, with consensus for 5' and 3' splice sites shown in *bold case*.

Figure 9. **Differential expression of GPBP and GPBP Δ 26.** Fragments representing the 78-bp exon (GPBP) or flanking sequences common to both isoforms (GPBP/GPBP Δ 26) were ³²P-labeled and used to hybridize human tissue and tumor cell line Northern blots (CLONTECH). The membranes were first hybridized with GPBP-specific probe, stripped and then reanalyzed with GPBP/GPBP Δ 26 probe. Washing conditions were less stringent for GPBP-specific probe (0.1 % SSPE, 37°C or 55°C) than for the GPBP/GPBP Δ 26 (0.1% SSPE, 68°C) to increase GPBP and GPBP Δ 26 signals respectively. No detectable signal was obtained for the GPBP probe when the washing program was at 68°C (not shown).

Figure 10. **GPBP Δ 26 displays lower phosphorylating activity than GPBP.** (A) Recombinantly-expressed, affinity purified GPBP (rGPBP) (lanes 1) or rGPBP Δ 26 (lanes 2) were subjected to SDS-PAGE under reducing conditions and either Coomassie blue stained (2 μ g per lane) or blotted (200ng per lane) with monoclonal antibodies recognizing the FLAG sequence (α -FLAG) or GPBP/GPBP Δ 26 (Mab14). (B) 200 ng of rGPBP (lanes 1) or rGPBP Δ 26 (lanes 2) were *in vitro* phosphorylated without substrate to assay auto-phosphorylation (left), or with 5 nmol GPpep1 to measure trans-phosphorylation activity (right). An arrowhead indicates the position of the peptide. (C) 3 μ g of rGPBP (lane 1) or rGPBP Δ 26 (lane 2) were in-blot renatured as described under Material and Methods. The numbers and bars indicate the molecular mass in kDa and the relative position of the molecular weight markers, respectively.

Figure 11. **rGPBP and rGPBP Δ 26 form very active high molecular weight aggregates.** About 300 μ g of rGPBP (A) or rGPBP Δ 26 (B) were subjected to gel filtration HPLC as described under Material and Methods. *Vertical arrowheads* and *numbers* respectively indicate the elution profile and molecular mass (kDa) of the molecular weight standards used. Larger aggregates eluted in the void volume (I), and the bulk of the material present in the samples eluted in the fractionation range of the column as a second peak between the 669 and 158 kDa markers (II). Fifteen microliters of the indicated minute fractions were subjected to SDS-PAGE and Coomassie blue staining. Five microliters of the same fractions were *in vitro* phosphorylated as described in Materials and Methods, and the reaction stopped by boiling in SDS sample buffer. The fractions were loaded onto SDS-PAGE, transferred to PVDF and autoradiographed for 1 or 2 hours using Kodak X-Omat films and blotted using anti-FLAG monoclonal antibodies (Sigma).

Figure 12. **Self-interaction of GPBP and GPBP Δ 26 assessed by a yeast two-hybrid system.** (A) Cell transfected for the indicated combinations of plasmids were selected on leucine-tryptophan-deficient medium (-*Trp*, -*Leu*), and independent transformants restreaked onto histidine-deficient plates (-*Trp*, -*Leu*, -*His*) in the presence or absence of 1 mM 3-amino-triazole (3-AT), to assess interaction. The picture was taken 3 days after streaking. (B) The bars represent mean values in β -galactosidase arbitrary units of four independent β -galactosidase in-solution assays.

Figure 13. **GPBP is expressed associated with endothelial and glomerular basement membranes.** Paraffin embedded sections of human muscle (A) or renal cortex (B, C) were probed with GPBP-specific antibodies (A,B) or with Mab189, a monoclonal antibody specific for the human α 3(IV)NC1 (C). Frozen sections of human kidney (D-F) were probed with Mab17, a monoclonal antibody specific for the α 3(IV)NC1 domain (D), GPBP-specific antibodies (E), or sera from a GP patient (F). Control sera (chicken pre-immune and human control) did not display tissue-binding in parallel studies (not shown).

Figure 14. **GPBP is expressed in human but not in bovine and murine renal cortex.** Cortex from human (A,

D), bovine (B, E) or murine (C, F) kidney were paraffin embedded and probed with either GPBP-specific antibodies (A-C) or GPBP/GPBP Δ 26-specific antibodies (D-F).

Figure 15. **GPBP is highly expressed in several autoimmune conditions.** Skeletal muscle total RNA from a control individual (lane 1) or from a GP patient (lane 2) was subjected to RT-PCR as in Fig.8, using the oligonucleotides 15m and 62c in the amplification program. Frozen (B-D) or paraffin embedded (E-G) human control skin (B, E) or skin affected by SLE (C, F) or lichen planus (D, G) were probed with GPBP-specific antibodies.

Figure 16. **Phosphorylation of GP alternative splicing products by PKA.** In left panel, equimolecular amounts of rGP (lanes 1), rGP Δ V (lanes 2), rGP Δ III (lanes 3) or rGP Δ III/IV/V (lanes 4), equivalent to 500 ng of the GP were phosphorylated at the indicated ATP concentrations. One-fifth of the total phosphorylation reaction mixture was separated by gel electrophoresis and transferred to PVDF, autoradiographed (shown) and the proteins blotted with M3/1, a specific monoclonal antibody recognizing all four species (shown) or using antibodies specific for each individual C-terminal region (not shown). Arrowheads indicate the position of each recombinant protein, from top to bottom, GP, GP Δ V and, GP Δ III -GP Δ III/IV/V which displayed the same mobilities. Right panel: purified α 3(IV)NC1 domain or hexamer was phosphorylated with PKA and 0.1 μ M ATP in the absence (lanes 1) or in the presence of 10 nmol of peptides representing the C-terminal region of either GP Δ III (lanes 2) or GP Δ III/IV/V (lanes 3). Where indicated the phosphorylation mixtures of purified α 3(IV)NC1 domain were V8 digested and immunoprecipitated with antibodies specific for the N terminus of the human α 3(IV)NC1 domain (3). Bars and numbers indicate the position and sizes (kDa) of the molecular weight markers.

Figure 17. **Sequence alignment of GP Δ III and MBP.** The phosphorylation sites for PKA (boxed) and the structural similarity for the sites at Ser 8 and 9 of MBP and GP Δ III respectively are shown (underlined). The identity (vertical bars) and chemical homology (dots) of the corresponding exon II (bent arrow) of both molecular species are indicated. The complete sequence of GP Δ III from the collagenase cleavage site (72-residues) is aligned with the 69-N terminal residues of MBP comprising the exon I and ten residues of the exon II.

Figure 18. **Phosphorylation of recombinant MBP proteins by PKA.** About 200 ng of rMBP (lane 1), or Ser to Ala mutants thereof in position 8 (lane 2) or 57 (lane 3), or rMPB Δ II (lane 4) or Ser to Ala mutants thereof in position 8 (lane 5) or 57 (lane 6), were phosphorylated by PKA and 0.1 μ M ATP. The mixtures were subjected to SDS-PAGE, transferred to PVDF and autoradiographed (Phosphorylation) and the individual molecular species blotted with monoclonal antibodies against human MBP obtained from Roche Molecular Biochemicals (Western).

Figure 19. **Phosphorylation of recombinant MBP proteins by GPBP.** About 200 ng of rMBP (lane 1), or Ser to Ala mutants thereof in positions 8 (lane 2) or 57 (lane 3), or rMPB Δ II (lane 4), or Ser to Ala mutants thereof in positions 8 (lane 5) or 57 (lane 6), were subjected to SDS-PAGE, transferred to PVDF, and the area containing the proteins visualized with Ponceau and stripped out. The immobilized proteins were *in situ* phosphorylated with rGPBP as described in Materials and Methods, autoradiographed (Phosphorylation) and subsequently blotted as in Fig. 18 (Western).

Figure 20. **Regulation of the GPBP by the C terminal region of GP Δ III.** About 200 ng of rGPBP were *in vitro* phosphorylated with 150 μ M ATP in the absence (lane 1) or in the presence of 5 mmol of GP Δ III-derived peptide synthesized either using Boc- (lane 2) or Fmoc- (lane 3) chemistry. The reaction mixtures were subjected to SDS-PAGE, transferred to PVDF and autoradiographed to assess autophosphorylation, and subsequently blotted with anti-FLAG monoclonal antibodies (Sigma) to determine the amount of recombinant material present (Western).

Detailed Description of the Invention

[0014] The abbreviations used herein are: bp, base pair; DTT, dithiothreitol; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene glycol-bis(β -aminoethyl ether) N,N,N',N'-tetraacetic acid; GP, Goodpasture; rGP Δ III, rGP Δ III/IV/V and rGP Δ V, recombinant material representing the alternative forms of the Goodpasture antigen resulting from splicing out exon III, exon III, IV and V or exon V, respectively; GPBP and rGPBP, native and recombinant Goodpasture antigen binding protein; GPBP Δ 26 and rGPBP Δ 26, native and recombinant alternative form of the GPBP; GST, glutathione S-transferase; HLA, human lymphocyte antigens; HPLC, high performance liquid chromatography; Kb, thousand base pairs; kDa, thousand daltons; MBP, rMBP, native and recombinant 21 kDa myelin basic protein; MBP Δ II and rMBP Δ II, native and recombinant 18.5 kDa myelin basic protein that results from splicing out exon II; MBP Δ V and MBP Δ II/V, myelin basic protein alternative forms resulting from splicing out exon V and exons II and V, respectively; MHC, major histocompatibility complex; NC1, non-collagenous domain; PH, pleckstrin homology; PKA, cAMP-dependent protein kinase; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl-sulfate polyacrylamide gel electrophoresis; TBS, tris buffered saline.

[0015] Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in *Methods in Enzymology* (M.P. Deutscher, ed., (1990)

Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, CA), *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney. 1987. Liss, Inc. New York, NY), *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX).

[0016] As used herein, the term "GPBP" refers to Goodpasture binding protein, and includes both monomers and oligomers thereof. Human (SEQ ID NO:2), mouse (SEQ ID NO:4), and bovine GPBP sequences (SEQ ID NO:6) are provided herein.

[0017] As used herein, the term "GPBP Δ 26" refers to Goodpasture binding protein deleted for the 26 amino acid sequence shown in SEQ ID NO:14, and includes both monomers and oligomers thereof. Human (SEQ ID NO:8), mouse (SEQ ID NO:10), and bovine GPBP sequences (SEQ ID NO:12) are provided herein.

[0018] As used herein the term "GPBP_{pep1}" refers to the 26 amino acid peptide shown in SEQ ID NO:14, and includes both monomers and oligomers thereof.

[0019] As used herein, the term "GP antigen" refers to the α 3 NC1 domain of type IV collagen.

[0020] As used herein, "MBP" refers to myelin basic protein.

[0021] In one aspect, the present invention provides isolated nucleic acids that encode GPBP, GPBP Δ 26, and GPBP_{pep1}, and mutants or fragments thereof. In one embodiment, the isolated nucleic acids comprise sequences substantially similar to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:25, or fragments thereof.

[0022] The phrase "substantially similar" is used herein in reference to the nucleotide sequence of DNA or RNA, or the amino acid sequence of protein, having one or more conservative or non-conservative variations from the disclosed sequences, including but not limited to deletions, additions, or substitutions, wherein the resulting nucleic acid and/or amino acid sequence is functionally equivalent to the sequences disclosed herein. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same protein disclosed herein. For example, functionally equivalent DNAs encode proteins that are the same as those disclosed herein or that have one or more conservative amino acid variations, such as substitution of a non-polar residue for another non-polar residue or a charged residue for a similarly charged residue. These changes include those recognized by those of skill in the art as substitutions that do not substantially alter the tertiary structure of the protein.

[0023] In practice, the term substantially similar means that DNA encoding two proteins hybridize to one another under conditions of moderate to high stringency, and encode proteins that have either the same sequence of amino acids, or have changes in sequence that do not alter their structure or function. As used herein, substantially similar sequences of nucleotides or amino acids share at least about 70% identity, more preferably at least about 80% identity, and most preferably at least about 90% identity. It is recognized, however, that proteins (and DNA or mRNA encoding such proteins) containing less than the above-described level of homology arising as splice variants or that are modified by conservative amino acid substitutions (or substitution of degenerate codons) are contemplated to be within the scope of the present invention.

[0024] Stringency of hybridization is used herein to refer to conditions under which nucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_M) of the hybrids. T_M decreases approximately 1-1.5°C with every 1% decrease in sequence homology. In general, the stability of a hybrid is a function of sodium ion concentration and temperature. Typically, the hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions. Thus, as used herein, moderate stringency refers to conditions that permit hybridization of those nucleic acid sequences that form stable hybrids in 0.1% SSPE at 37°C or 55°C, while high stringency refers to conditions that permit hybridization of those nucleic acid sequences that form stable hybrids in 0.1% SSPE at 65°C. It is understood that these conditions may be duplicated using a variety of buffers and temperatures and that they are not necessarily precise. Denhardt's solution and SSPE (see, e.g., Sambrook, Fritsch, and Maniatis, in: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1989) are well known to those of skill in the art, as are other suitable hybridization buffers.

[0025] The isolated nucleic acid sequence may comprise an RNA, a cDNA, or a genomic clone with one or more introns. The isolated sequence may further comprise additional sequences useful for promoting expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals.

[0026] In another aspect, the present invention provides recombinant expression vectors comprising nucleic acid sequences that express GPBP, GPBP Δ 26, or GPBP_{pep1}, and mutants or fragments thereof. In one embodiment, the vectors comprise nucleic acid sequences that are substantially similar to the sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:25, or fragments thereof.

[0027] "Recombinant expression vector" includes vectors that operatively link a nucleic acid coding region or gene to

any promoter capable of effecting expression of the gene product. The promoter sequence used to drive expression of the disclosed nucleic acid sequences in a mammalian system may be constitutive (driven by any of a variety of promoters, including but not limited to, CMV, SV40, RSV, actin, EF) or inducible (driven by any of a number of inducible promoters including, but not limited to, tetracycline, ecdysone, steroid-responsive). The construction of expression vectors for use

5 in transfecting prokaryotic cells is also well known in the art, and thus can be accomplished via standard techniques. (See, for example, Sambrook, Fritsch, and Maniatis, in: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1989; *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray. The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX)

10 **[0028]** The expression vector must be replicable in the host organisms either as an episome or by integration into host chromosomal DNA. In a preferred embodiment, the expression vector comprises a plasmid. However, the invention is intended to include other expression vectors that serve equivalent functions, such as viral vectors.

[0029] In a further aspect, the present invention provides host cells that have been transfected with the recombinant expression vectors disclosed herein, wherein the host cells can be either prokaryotic or eukaryotic. The cells can be transiently or stably transfected. Such transfection of expression vectors into prokaryotic and eukaryotic cells can be

15 accomplished via any technique known in the art, including but not limited to standard bacterial transformations, calcium phosphate co-precipitation, electroporation, or liposome mediated-, DEAE dextran mediated-, polycationic mediated-, or viral mediated transfection. (See, for example, *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press; *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.L Freshney. 1987. Liss, Inc. New York, NY),

20 **[0030]** In a still further aspect, the present invention provides substantially purified GPBP, GPBP Δ 26, and GPBPpep1, and mutants or fragments thereof. In one embodiment, the amino acid sequence of the substantially purified protein is substantially similar to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or peptide fragments thereof.

[0031] As used herein, the term "substantially purified" means that the protein has been separated from its in vivo cellular environments. Thus, the protein can either be purified from natural sources, or recombinant protein can be purified from the transfected host cells disclosed above. In a preferred embodiment, the proteins are produced by the transfected cells disclosed above, and purified using standard techniques. (See for example, *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press.)) The protein can thus be purified from prokaryotic or eukaryotic sources. In various further preferred embodiments, the protein is purified from bacterial,

25 yeast, or mammalian cells.

[0032] The protein may comprise additional sequences useful for promoting purification of the protein, such as epitope tags and transport signals. Examples of such epitope tags include, but are not limited to FLAG (Sigma Chemical, St. Louis, MO), myc (9E10) (Invitrogen, Carlsbad, CA), 6-His (Invitrogen; Novagen, Madison, WI), and HA (Boehringer Manheim Biochemicals). Examples of such transport signals include, but are not limited to, export signals, secretory

30 signals, nuclear localization signals, and plasma membrane localization signals.

[0033] In another aspect, the present invention provides antibodies that selectively bind to GPBP, GPBP Δ 26, or GPBPpep1. In one aspect, the antibodies selectively bind to a protein comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or peptide fragments thereof. Such antibodies can be

35 produced by immunization of a host animal with either the complete GPBP, or with antigenic peptides thereof. The antibodies can be either polyclonal or monoclonal.

[0034] Antibodies can be made by well-known methods, such as described in Harlow and Lane, *Antibodies; A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (1988). In one example, preimmune serum is collected prior to the first immunization. Substantially purified proteins of the invention, or antigenic fragments thereof,

40 together with an appropriate adjuvant, is injected into an animal in an amount and at intervals sufficient to elicit an immune response. Animals are bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. At about 7 days after each booster immunization, or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20° C. Polyclonal antibodies against the proteins and peptides of the invention can then be purified directly by passing

45 serum collected from the animal through a column to which non-antigen-related proteins prepared from the same expression system without GPBP-related proteins bound.

50 **[0035]** Monoclonal antibodies can be produced by obtaining spleen cells from the animal. (See Kohler and Milstein, *Nature* 256, 495-497 (1975)). In one example, monoclonal antibodies (mAb) of interest are prepared by immunizing inbred mice with the proteins or peptides of the invention, or an antigenic fragment thereof. The mice are immunized by

55 the IP or SC route in an amount and at intervals sufficient to elicit an immune response. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of by the intravenous (IV) route. Lymphocytes, from antibody positive mice are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the

splenic lymphocytes with an appropriate fusion partner under conditions which will allow the formation of stable hybridomas. The antibody producing cells and fusion partner cells are fused in polyethylene glycol at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells and are screened for antibody production by an immunoassay such as solid phase immunoradioassay. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, *Soft Agar Techniques*, in *Tissue Culture Methods and Applications*, Kruse and Paterson, Eds., Academic Press, 1973.

[0036] To generate such an antibody response, the proteins of the present invention are typically formulated with a pharmaceutically acceptable carrier for parenteral administration. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The formulation of such compositions, including the concentration of the polypeptide and the selection of the vehicle and other components, is within the skill of the art.

[0037] The term antibody as used herein is intended to include antibody fragments thereof which are selectively reactive with the proteins and peptides of the invention, or fragments thereof. Antibodies can be fragmented using conventional techniques, and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab')₂ fragments can be generated by treating antibody with pepsin. The resulting F(ab')₂ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

[0038] In a further aspect, the invention provides methods for detecting the presence of the proteins or peptides of the invention in a protein sample, comprising providing a protein sample to be screened, contacting the protein sample to be screened with an antibody against the proteins or peptides of the invention, and detecting the formation of antibody-antigen complexes. The antibody can be either polyclonal or monoclonal as described above, although monoclonal antibodies are preferred. As used herein, the term "protein sample" refers to any sample that may contain the proteins or peptides of the invention, and fragments thereof, including but not limited to tissues and portions thereof, tissue sections, intact cells, cell extracts, purified or partially purified protein samples, bodily fluids, nucleic acid expression libraries. Accordingly, this aspect of the present invention may be used to test for the presence of GPBP, GPBP Δ 26, GPBPpep1, or alternative products of the GP antigen in these various protein samples by standard techniques including, but not limited to, immunolocalization, immunofluorescence analysis, Western blot analysis, ELISAs, and nucleic acid expression library screening, (See for example, Sambrook et al, 1989.) In one embodiment, the techniques may determine only the presence or absence of the protein or peptide of interest. Alternatively, the techniques may be quantitative, and provide information about the relative amount of the protein or peptide of interest in the sample. For quantitative purposes, ELISAs are preferred.

[0039] Detection of immunocomplex formation between the proteins or peptides of the invention, or fragments thereof, and their antibodies or fragments thereof, can be accomplished by standard detection techniques. For example, detection of immunocomplexes can be accomplished by using labeled antibodies or secondary antibodies. Such methods, including the choice of label are known to those ordinarily skilled in the art. (Harlow and Lane, *Supra*). Alternatively, the polyclonal or monoclonal antibodies can be coupled to a detectable substance. The term "coupled" is used to mean that the detectable substance is physically linked to the antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase. Examples of suitable prosthetic-group complexes include streptavidin/biotin and avidin/biotin. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin. An example of a luminescent material includes luminol. Examples of suitable radioactive material include ¹²⁵I, ¹³¹I, ³⁵S or ³H.

[0040] Such methods of detection are useful for a variety of purposes, including but not limited to detecting an autoimmune condition, identifying cells targeted for or undergoing apoptosis, immunolocalization of the proteins of interest in a tissue sample, Western blot analysis, and screening of expression libraries to find related proteins.

[0041] In yet another aspect, the invention provides methods for detecting the presence in a sample of nucleic acid sequences encoding the GPBP, GPBP Δ 26, GPBPpep1, or alternative products of the GP antigen comprising providing a nucleic acid sample to be screened, contacting the sample with a nucleic acid probe derived from the isolated nucleic acid sequences of the invention, or fragments thereof, and detecting complex formation.

[0042] As used herein, the term "sample" refers to any sample that may contain GPBP-related nucleic acid, including but not limited to tissues and portions thereof, tissue sections, intact cells, cell extracts, purified or partially purified nucleic acid samples, DNA libraries, and bodily fluids. Accordingly, this aspect of the present invention may be used to test for the presence of GPBP mRNA or DNA in these various samples by standard techniques including, but not limited to, *in situ* hybridization, Northern blotting, Southern blotting, DNA library screening, polymerase chain reaction (PCR) or reverse transcription-PCR (RT-PCR). (See for example, Sambrook et al, 1989.) In one embodiment, the techniques may determine only the presence or absence of the nucleic acid of interest. Alternatively, the techniques may be quan-

titative, and provide information about the relative amount of the nucleic acid of interest in the sample. For quantitative purposes, quantitative PCR and RT-PCR are preferred. Thus, in one example, RNA is isolated from a sample, and contacted with an oligonucleotide derived from the nucleic acid sequence of interest, together with reverse transcriptase under suitable buffer and temperature conditions to produce cDNAs from the GPBP-related RNA. The cDNA is then subjected to PCR using primer pairs derived from the nucleic acid sequence of interest. In a preferred embodiment, the primers are designed to detect the presence of the RNA expression product of SEQ ID NO:5, and the amount of GPBP gene expression in the sample is compared to the level in a control sample.

[0043] For detecting the nucleic acid sequence of interest, standard labeling techniques can be used to label the probe, the nucleic acid of interest, or the complex between the probe and the nucleic acid of interest, including, but not limited to radio-, enzyme-, chemiluminescent-, or avidin or biotin-labeling techniques, all of which are well known in the art. (See, for example, *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, CA)).

[0044] Such methods of nucleic acid detection are useful for a variety of purposes, including but not limited to diagnosing an autoimmune condition, identifying cells targeted for or undergoing apoptosis, in situ hybridization, Northern and Southern blot analysis, and DNA library screening.

[0045] As demonstrated in the following examples, GPBP shows preferential expression in tissue structures that are commonly targeted in naturally-occurring autoimmune responses, and is highly expressed in several autoimmune conditions, including but not limited to Goodpasture Syndrome (GP), systemic lupus erythematosus (SLE), and lichen planus. Furthermore, following a similar experimental approach to that described below, recombinant proteins representing autoantigens in GP disease ($\alpha 3$ Type IV collagen), SLE (P1 ribosomal phosphoprotein and Sm-D1 small nuclear ribonucleoproteins) and dermatomyositis (hystidyl-tRNA synthetase) were shown to be in vitro substrates of GPBP.

[0046] Thus, in a preferred embodiment, detection of GPBP expression is used to detect an autoimmune condition. A sample that is being tested is compared to a control sample for the expression of GPBP, wherein an increased level of GPBP expression indicates the presence of an autoimmune condition. In this embodiment, it is preferable to use antibodies that selectively bind to GPBP_{pep1}, which is present in GPBP but not in GPBP $\Delta 26$.

[0047] Furthermore, as shown in the accompanying examples, GPBP is down-regulated in tumor cell lines, and the data suggest that GPBP/GPBP $\Delta 26$ are likely to be involved in cell signaling pathways that induce apoptosis, which may be up-regulated during autoimmune pathogenesis and down-regulated during cell transformation to prevent autoimmune attack to transformed cells during tumor growth. Thus, the detection methods disclosed herein can be used to detect cells that are targeted for, or are undergoing apoptosis.

[0048] In another aspect, the present invention provides a method for treating an autoimmune disorder, a tumor, or for preventing cell apoptosis comprising modification of the expression or activity of GPBP, GPBP $\Delta 26$, or a protein comprising a polypeptide substantially similarly to GPBP_{pep1} in a patient in need thereof. Modifying the expression or activity of GPBP, GPBP $\Delta 26$, or a protein comprising a polypeptide substantially similarly to GPBP_{pep1} can be accomplished by using specific inducers or inhibitors of GPBP expression or activity, GPBP antibodies, gene or protein therapy using GP or myelin basic protein alternative products, cell therapy using host cells expressing GP or myelin basic protein alternative products, antisense therapy, or other techniques known in the art. In a preferred embodiment, the method further comprises administering a substantially purified alternative product of the GP antigen or MBP to modify the expression or activity of GPBP, GPBP $\Delta 26$, or a protein comprising a polypeptide substantially similarly to GPBP_{pep1}. As used herein, "modification of expression or activity" refers to modifying expression or activity of either the RNA or protein product.

[0049] In a further aspect, pharmaceutical compositions, comprising an amount effective of substantially purified alternative products of the GP antigen or MBP to modify the expression or activity of GPBP RNA or protein, and a pharmaceutically acceptable carrier, may be used.

[0050] For administration, the active agent is ordinarily combined with one or more adjuvants appropriate for the indicated route of administration. The compounds may be mixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the compounds of this invention may be dissolved in saline, water, polyethylene glycol, propylene glycol, carboxymethyl cellulose colloidal solutions, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

[0051] The present invention may be better understood with reference to the accompanying examples that are intended for purposes of illustration only and should not be construed to limit the scope of the invention, as defined by the claims appended hereto.

Example 1: Characterization of GPBP

[0052] Here we report the cloning and characterization of a novel type of serine/threonine kinase that specifically binds to and phosphorylates the unique N-terminal region of the human GP antigen.

MATERIALS AND METHODS

[0053] Synthetic polymers-Peptides. GPpep1, KGKRGDSGSPATWTTRGFVFT (SEQ ID NO:26), representing residues 3-23 of the human GP antigen and GPpep1Ala⁹, KGKRGDAGSPATWTTRGFVFT (SEQ ID NO:27), a mutant Ser⁹ to Ala⁹ thereof, were synthesized by MedProbe and CHIRON. FLAG peptide, was from Sigma.

[0054] Oligonucleotides. The following as well as several other GPBP-specific oligonucleotides were synthesized by Genosys and GIBCO BRL:

ON-GPBP-54m:

TCGAATTCACCATGGCCCCACTAGCCGACTACAAGGACGACGATG
ACAAG (SEQ ID NO: 28).

ON-GPBP-55c:

CCGAGCCCGACGAGTTCCAGCTCTGATTATCCGACATCTTGTCATCG
TCG (SEQ ID NO:29).

ON-HNC-B-N-14m: CGGGATCCGCTAGCTAAGCCAGGCAAGGATGG (SEQ ID
NO:30).

ON-HNC-B-N-16c: CGGGATCCATGCATAAATAGCAGTTCTGCTGT (SEQ ID
NO:31).

[0055] Isolation and characterization of cDNA clones encoding human GPBP-Several human λ -gt11 cDNA expression libraries (eye, fetal and adult lung, kidney and HeLa S3, from CLONTECH) were probed for cDNAs encoding proteins interacting with GPpep1. Nitrocellulose filters (Millipore) prepared following standard immunoscreening procedures were blocked and incubated with 1-10 nmoles per ml of GPpep1 at 37°C. Specifically bound GPpep1 was detected using M3/1A monoclonal antibodies (7). A single clone was identified in the HeLa-derived library (HeLa1). Specificity of fusion protein binding was confirmed by similar binding to recombinant eukaryotic human GP antigen. The EcoRI cDNA insert of HeLa1 (0.5-kb) was used to further screen the same library and to isolate overlapping cDNAs. The largest cDNA (2.4-kb) containing the entire cDNA of HeLa1 (n4') was fully sequenced.

[0056] Northern and Southern blots-Pre-made Northern and Southern blots (CLONTECH) were probed with HeLa1 cDNA following manufacturer instructions.

[0057] Plasmid construction, expression and purification of recombinant proteins-GPBP-derived material. The original λ -gt11 HeLa1 clone was expressed as a lysogen in E. Coli Y1089 (8). The corresponding β -galactosidase-derived fusion protein containing the N-terminal 150 residues of GPBP was purified from the cell lysate using an APTG-agarose column (Boehringer). The EcoRI 2.4-kb fragment of n4' was subcloned in Bluescribe M13+ vector (Stratagene) (BS-n4'), amplified and used for subsequent cloning. A DNA fragment containing (from 5' to 3'), an EcoRI restriction site, a standard Kozak consensus for translation initiation, a region coding for a tag peptide sequence (FLAG, DYKDDDDK (SEQ ID NO:32)), and the sequence coding for the first eleven residues of GPBP including the predicted Met₁ and a Ban II restriction site, was obtained by hybridizing ON-GPBP-54m and ON-GPBP-55c, and extending with modified T₇ DNA polymerase (Amersham). The resulting DNA product was digested with EcoRI and BanII, and ligated with the BanII/EcoRI cDNA fragment of BS-n4' in the EcoRI site of pHIL-D2 (Invitrogen) to produce pHIL-FLAG-n4'. This plasmid was used to obtain Mut^s transformants of the GS115 strain of *Pichia pastoris* and to express FLAG-tagged recombinant GPBP

(rGPBP) either by conventional liquid culture or by fermentation procedures (*Pichia* Expression Kit, Invitrogen). The cell lysates were loaded onto an anti-FLAG M2 column (Sigma), the unbound material washed out with Tris buffered saline (TBS, 50 mM Tris-HCl, pH 7.4, 150 mM NaCl) or salt-supplemented TBS (up to 2M NaCl), and the recombinant material eluted with FLAG peptide. For expression in cultured human kidney-derived 293 cells (ATCC 1573-CRL), the 2.4- or 2.0-kb EcoRI cDNA insert of either BS-n4' or pHIL-FLAG-n4' was subcloned in pcDNA3 (Invitrogen) to produce pc-n4' and pc-FLAG-n4' respectively. When used for transient expression, 18 hours after transfection the cells were lysed with 3.5-4 $\mu\text{l}/\text{cm}^2$ of chilled lysis buffer (1% Nonidet P-40 or Triton-X100, 5mM EDTA and 1 mM PMSF in TBS) with or without 0.1% SDS, depending on whether the lysate was to be used for SDS-PAGE or FLAG-purification, respectively. For FLAG purification, the lysate of four to six 175 cm^2 culture dishes was diluted up to 50 ml with lysis buffer and purified as above. For stable expression, the cells were similarly transfected with pc-n4' and selected for three weeks with 800 $\mu\text{g}/\text{ml}$ of G418. For bacterial recombinant expression, the 2.0-kb EcoRI cDNA fragment of pHIL-FLAG-n4' was cloned in-frame downstream of the glutathione S-transferase (GST)-encoding cDNA of pGEX-5x-1 (Pharmacia). The resulting construct was used to express GST-GPBP fusion protein in DH5 α cells (9).

[0058] GP antigen-derived material. Human recombinant GP antigen (rGP) was produced in 293 cells using the pRc/CMV-BM40 expression vector containing the α 3-specific cDNA between ON-HNC-B-N-14m and ON-HNC-B-N-16c. The expression vector is a pRc/CMV (Invitrogen)-derived vector provided by Billy G. Hudson (Kansas University Medical Center) that contains cDNA encoding an initiation Met, a BM40 signal peptide followed by a tag peptide sequence (FLAG), and a polylinker cloning site. To obtain α 3-specific cDNA, a polymerase chain reaction was performed using the oligonucleotides above and a plasmid containing the previously reported α 3(IV) cDNA sequence (3) as template (clone C2). For stable expression of rGP, 293 cells were transfected with the resulting construct (α 3VLC) and selected with 400 $\mu\text{g}/\text{ml}$ of G418. The harvested rGP was purified using an anti-FLAG M2 column.

[0059] All the constructs were verified by restriction mapping and nucleotide sequencing.

[0060] Cell culture and DNA transfection-Human 293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Transfections were performed using the calcium phosphate precipitation method of the Profection Mammalian Transfection Systems (Promega). Stably transfected cells were selected by their resistance to G418. Foci of surviving cells were isolated, cloned and amplified.

[0061] Antibody production-Polyclonal antibodies against the N-terminal region of GPBP. Cells expressing HeLa1 λ -gt11 as a lysogen were lysed by sonication in the presence of Laemmli sample buffer and subjected to electrophoresis in a 7.5% acrylamide preparative gel. The gel was stained with Coomassie blue and the band containing the fusion protein of interest excised and used for rabbit immunization (10). The anti-serum was tested for reactivity using APTG-affinity purified antigen. To obtain affinity-purified antibodies, the anti-serum was diluted 1:5 with TBS and loaded onto a Sepharose 4B column containing covalently bound affinity purified antigen. The bound material was eluted and, unless otherwise indicated, used in the immunochemical studies.

[0062] Monoclonal antibodies against GPBP. Monoclonal antibodies were produced essentially as previously reported (7) using GST-GPBP. The supernatants of individual clones were analyzed for antibodies against rGPBP.

[0063] In vitro phosphorylation assays-About 200 ng of rGPBP were incubated overnight at 30°C in 25 mM β -glycerolphosphate (pH 7.0), 0.5 mM EDTA, 0.5 mM EGTA, 8 mM MgCl_2 , 5 mM MnCl_2 , 1 mM DTT and 0.132 μM γ - ^{32}P -ATP, in the presence or absence of 0.5-1 μg of protein substrates or 10 nmoles of synthetic peptides, in a total volume of 50 μl .

[0064] In vivo phosphorylation assays-Individual wells of a 24-well dish were seeded with normal or with stably pc-n4' transfected 293 cells. When the cells were grown to the desired density, a number of wells of the normal 293 cells were transfected with pc-FLAG-n4'. After 12 hours, the culture medium was removed, 20 $\mu\text{Ci}/\text{well}$ of H_3 $^{32}\text{PO}_4$ in 100 μl of phosphate-free DMEM added, and incubation continued for 4 hours. The cells were lysed with 300 $\mu\text{l}/\text{well}$ of TBS containing 1% Triton X-100, 2 mM EDTA, 1 mM PMSF, 50 mM NaF and 0.2 mM vanadate, and extracted with specific antibodies and Protein A-Sepharose. When anti-GPBP serum was used, the lysate was pre-cleared using pre-immune serum and Protein A-Sepharose.

[0065] In vitro dephosphorylation of rGPBP-About 1 μg of rGPBP was dephosphorylated in 100 μl of 10 mM Tris-acetate (pH 7.5), 10 mM magnesium acetate and 50 mM potassium acetate with 0.85 U of calf intestine alkaline phosphatase (Pharmacia) for 30 min at 30°C.

[0066] Renaturation assays-In-blot renaturation assays were performed using 1-5 μg of rGPBP as previously described (11).

[0067] Nucleotide sequence analysis- cDNA sequence analyses were performed by the dideoxy chain termination method using [α] ^{35}S -dATP, modified T₇ DNA polymerase (Amersham) and universal or GPBP-specific primers (8-10).

[0068] ^{32}P -Phosphoamino acid analysis-Immunopurified rGPBP or HPLC gel-filtration fractions thereof containing the material of interest were phosphorylated, hydrolyzed and analyzed in one dimensional (4) or two dimensional thin layer chromatography (12). When performing two dimensional analysis, the buffer for the first dimension was formic acid:acetic acid:water (1:3.1:35.9) (pH 1.9) and the buffer for the second dimension was acetic acid:pyridine:water (2:0.2:37.8) (pH 3.5). Amino acids were revealed with ninhydrin, and ^{32}P -phosphoamino acids by autoradiography.

[0069] Physical methods and immunochemical techniques-SDS-PAGE and Western-blotting were performed as

in (4). Immunohistochemistry studies were done on human multi-tissue control slides (Biomeda, Biogenex) using the ABC peroxidase method (13).

[0070] Computer analysis-Homology searches were carried out against the GenBank and SwissProt databases with the BLAST 2.0 (14) at the NCBI server, and against the TIGR Human Gene Index database for expressed sequence tags, using the Institute for Genomic Research server. The search for functional patterns and profiles was performed against the PROSITE database using the ProfileScan program at the Swiss Institute of Bioinformatics (15). Prediction of coiled-coil structures was done at the Swiss Institute for Experimental Cancer Research using the program Coils (16) with both 21 and 28 residue windows.

RESULTS

[0071] Molecular cloning of GPBP-To search for proteins specifically interacting with the divergent N-terminal region of the human GP antigen, a 21-residue peptide (GPpеп1; SEQ ID NO:26), encompassing this region and flanking sequences, and specific monoclonal antibodies against it were combined to screen several human cDNA expression libraries. More than 5×10^6 phages were screened to identify a single HeLa-derived recombinant encoding a fusion protein specifically interacting with GPpеп1 without disturbing antibody binding.

[0072] Using the cDNA insert of the original clone (HeLa1), we isolated a 2.4-kb cDNA (n4') that contains 408-bp of 5'-untranslated sequence, an open reading frame (ORF) of 1872-bp encoding 624 residues, and 109-bp of 3'-untranslated sequence (Fig. 1) (SEQ ID NO:1-2). Other structural features are of interest. First, the predicted polypeptide (hereinafter referred to as GPBP) has a large number of phosphorylatable (17.9%) and acidic (16%) residues unequally distributed along the sequence. Serine, which is the most abundant residue (9.3%), shows preference for two short regions of the protein, where it comprises nearly 40% of the amino acids, compared to an average of less than 7% throughout the rest of the polypeptide chain. It is also noteworthy that the more N-terminal, serine-rich region consists mainly of a Ser-Xaa-Yaa repeat. Acidic residues are preferentially located at the N-terminal three-quarters of the polypeptide, with nearly 18% of the residues being acidic. These residues represent only 9% in the most C-terminal quarter of the polypeptide, resulting in a polypeptide chain with two electrically opposite domains. At the N-terminus, the polypeptide contains a pleckstrin homology (PH) domain, which has been implicated in the recruitment of many signaling proteins to the cell membrane where they exert their biological activities (17). Finally, a bipartite nuclear targeting sequence (18) exists as an integral part of a heptad repeat region that meets all the structural requirements to form a coiled-coil (16).

[0073] Protein data bank searches revealed homologies almost exclusively within the approximately 100 residues at the N-terminal region harboring the PH domain. The PH domain of the oxysterol-binding protein is the most similar, with an overall identity of 33.5% and a similarity of 65.2% with GPBP. In addition, the *Caenorhabditis elegans* cosmid F25H2 (accession number Q93569) contains a hypothetical ORF that displays an overall identity of 26.5% and a similarity of 61% throughout the entire protein sequence, indicating that similar proteins are present in lower invertebrates. Several human expressed sequence tags (accession numbers AA287878, AA287561, AA307431, AA331618, AA040134, AA158618, AA040087, AA122226, AA158617, AA121104, AA412432, AA412433, AA282679 and N27578) possess a high degree of nucleotide identity (above 98%) with the corresponding stretches of the GPBP cDNA, suggesting that they represent human GPBP. Interestingly, the AA287878 EST shows a gap of 67 nucleotides within the sequence corresponding to the GPBP 5'-untranslated region, suggesting that the GPBP pre-mRNA is alternatively spliced in human tissues (not shown).

[0074] The distribution and expression of the GPBP gene in human tissues was first assessed by Northern blot analysis (Fig. 2, panel A). The gene is expressed as two major mRNAs species between 4.4-kb and 7.5-kb in length and other minor species of shorter lengths. The structural relationship between these multiple mRNA species is not known and their relative expression varies between tissues. The highest expression level is seen in striated muscle (skeletal and heart), while lung and liver show the lowest expression levels.

[0075] Southern blot studies analysis of genomic DNA from different species indicated that homologous genes exist throughout phylogeny (Fig. 2, panel B). Consistent with the human origin of the probe, the hybridization intensities decreased in a progressive fashion as the origin of the genomic DNA moves away from humans in evolution.

[0076] Experimental determination of the translation start site-To experimentally confirm the predicted ORF, eukaryotic expression vectors containing either the 2.4-kb of cDNA of n4', or only the predicted ORF tagged with a FLAG sequence (Fig. 3A), were used for transient expression assays in 293 cells. The corresponding extracts were analyzed by immunoblot using GPBP- or FLAG-specific antibodies. The GPBP-specific antibodies bind to a similar major polypeptide in both transfected cells, but only the polypeptide produced by the engineered construct expressed the FLAG sequence (Fig. 3B). This located the translation start site of the n4' cDNA at the predicted Met and confirmed the proposed primary structure. Furthermore, the recombinant polypeptides displayed a molecular mass higher than expected (80 versus 71 kDa) suggesting that GPBP undergoes post-translational modifications.

[0077] Expression and characterization of yeast rGPBP-Yeast expression and FLAG-based affinity-purification were combined to produce rGPBP (Fig. 4A). A major polypeptide of ~89 kDa, along with multiple related products

displaying lower M_r , were obtained. The recombinant material was recognized by both anti-FLAG and GPBP-specific antibodies, guaranteeing the fidelity of the expression system. Again, however, the M_r displayed by the major product was notably higher than predicted and even higher than the M_r of the 293 cell-derived recombinant material, supporting the idea that GPBP undergoes important and differential post-translational modifications. Since phosphorylatable residues are abundant in the polypeptide chain, we investigated the existence of phosphoamino acids in the recombinant materials. By using monoclonal or polyclonal (not shown) antibodies against phosphoserine (Pser), phosphothreonine (PThr) and phosphotyrosine (PTyr), we identified the presence of all three phosphoresidues either in yeast rGPBP (Fig. 4B) or in 293 cell-derived material (not shown). The specificity of the antibodies was further assessed by partially inhibiting their binding by the addition of 5-10 mM of the corresponding phosphoamino acid (not shown). This suggests that the phosphoresidue content varies depending upon the cell expression system, and that the M_r differences are mainly due to phosphorylation. Dephosphorylated yeast-derived material consistently displayed similar M_r to the material derived from 293 cells, and phosphoamino acid content correlates with SDS-PAGE mobilities (Fig.4C). As an *in vivo* measurement, the phosphorylation of rGPBP in the 293 cells was assessed (Fig. 4D). Control cells (lanes 1) and cells expressing rGPBP in a stable (lanes 2) or transient (lanes 3) mode were cultured in the presence of $H_3^{32}PO_4$. Immunoprecipitated recombinant material contained ^{32}P , indicating that phosphorylation of GPBP occurred *in vivo* and therefore is likely to be a physiological process.

[0078] The rGPBP is a serine/threonine kinase that phosphorylates the N-terminal region of the human GP antigen-Although GPBP does not contain the conserved structural regions required to define the classic catalytic domain for a protein kinase, the recent identification and characterization of novel non-conventional protein kinases (19-27) encouraged the investigation of its phosphorylating activity. Addition of $[\gamma^{32}P]ATP$ to rGPBP (either from yeast or 293 cells (not shown)) in the presence of Mn^{2+} and Mg^{2+} resulted in the incorporation of ^{32}P as Pser and PThr in the major and related products recognized by both anti-FLAG and specific antibodies (Fig. 5A and B), indicating that the affinity-purified material contains a Ser/Thr protein kinase. To further characterize this activity, GPpep1, GPpep1Ala⁹ (a GPpep1 mutant with Ser⁹ replaced by Ala), native and recombinant human GP antigens, and native bovine GP antigen were assayed (Fig. 5C). Affinity-purified rGPBP phosphorylates all human-derived material to a different extent. However, in similar conditions, no appreciable ^{32}P -incorporation was observed in the bovine-derived substrate. The lower ^{32}P incorporation displayed by GPpep1Ala⁹ when compared with GPpep1, and the lack of phosphorylation of the bovine antigen, indicates that the kinase present in rGPBP discriminates between human and bovine antigens, and that Ser⁹ is a target for the kinase.

[0079] Although the purification system provides high quality material, the presence of contaminants with a protein kinase activity could not be ruled out. The existence of contaminants was also suggested by the presence of a FLAG-containing 40 kDa polypeptide, which displayed no reactivity with specific antibodies nor incorporation of ^{32}P in the phosphorylation assays (Fig. 4A and 5A). To precisely identify the polypeptide harboring the protein kinase activity, we performed *in vitro* kinase renaturation assays after SDS-PAGE and Western-blotted (Fig. 6). We successfully combined the use of specific antibodies (lane 1) and autoradiographic detection of *in situ* ^{32}P -incorporation (lane 2), and identified the 89 kDa rGPBP material as the primary polypeptide harboring the Ser/Thr kinase activity. The lack of ^{32}P -incorporation in the rGPBP-derived products, as well as in the 40 kDa contaminant, further supports the specificity of the renaturation assays and locates the kinase activity to the 89 kDa polypeptide. Recently, it has been shown that traces of protein kinases intimately associated with a polypeptide can be released from the blot membrane, bind to, and phosphorylate the polypeptide during the labeling step (28). To assess this possibility in our system, we performed renaturation studies using a small piece of membrane containing the 89 kDa polypeptide, either alone or together with membrane pieces representing the different regions of the blot lane. We observed similar ^{32}P -incorporation at the 89 kDa polypeptide regardless of the co-incubated pieces (not shown), indicating that if there are co-purified protein kinases in our sample they are not phosphorylating the 89 kDa polypeptide in the renaturation assays unless they co-migrate. Co-migration does not appear to be a concern, however, since rGPBP deletion mutants (GPBP Δ 26 and R3; see below) displaying different mobilities also have kinase activities and could be similarly in-blot renatured (not shown).

[0080] Immunohistochemical localization of the novel kinase-To investigate GPBP expression in human tissues we performed immunohistochemical studies using specific polyclonal (Fig.7) or monoclonal antibodies (not shown). Although GPBP is widely expressed in human tissues, it shows tissue and cell-specificity. In kidney, the major expression is found at the tubule epithelial cells and the glomerular mesangial cells and podocytes. At the lung alveolus, the antibodies display a linear pattern suggestive of a basement membrane localization, along with staining of pneumocytes. Liver shows low expression in the parenchyma, but high expression in biliary ducts. Expression in the central nervous system is observed in the white matter, but not in the neurons of the brain. In testis, a high expression in the spermatogonium contrasts with the lack of expression in Sertoli cells. The adrenal gland shows a higher level of expression in cortical cells versus the medullar. In the pancreas, GPBP is preferentially expressed in Langerhans islets versus the exocrine moiety. In prostate, GPBP is expressed in the epithelial cells but not in the stroma (Fig. 7). Other locations with high expression of GPBP are striated muscle, epithelial cells of intestinal tract, and Purkinje cells of the cerebellum (not shown). In general, in tissues where GPBP is highly expressed the staining pattern is mainly diffuse cytosolic. However

in certain locations there is, in addition, an important staining reinforcement at the nucleus (spermatogonium), at the plasma membrane (pneumocyte, hepatocyte, prostate epithelial cells, white matter) or at the extracellular matrix (alveolus) (Fig. 7).

5 DISCUSSION

[0081] Our data show that GPBP is a novel, non-conventional serine/threonine kinase. We also present evidence that GPBP discriminates between human and bovine GP antigens, and targets the phosphorylatable region of human GP antigen *in vitro*. Several lines of evidence indicate that the 89 kDa polypeptide is the only kinase in the affinity purified rGPBP. First, we found no differences in auto- or trans-phosphorylation among rGPBP samples purified in the presence of 150 mM, 0.5 M, 1 M or 2 M salt (not shown), suggesting that rGPBP does not carry intimately bound kinases. Second, there is no FLAG-containing, yeast-derived kinase in our samples, since material purified using GPBP-specific antibodies shows no differences in phosphorylation (not shown). Third, a deletion mutant (GPBP Δ 26; see below) displays reduced auto- and trans-phosphorylation activities (not shown), demonstrating that the 89 kD polypeptide is the only portion of the rGPBP with the ability to carry out phosphate transfer.

[0082] Although GPBP is not homologous to other non-conventional kinases, they share some structural features including an N-terminal α -helix coiled-coil (26, 27), serine-rich motifs (24), high phosphoamino acids content (27), bipartite nuclear localization signal (27), and the absence of a typical nucleotide or ATP binding motif (24, 27).

[0083] Immunohistochemistry studies show that GPBP is a cytosolic polypeptide also found in the nucleus, associated with the plasma membrane and likely at the extracellular matrix associated with the basement membrane, indicating that it contains the structural requirements to reach all these destinations. The nuclear localization signal and the PH domain confer to it the potential to reach the nucleus and the cell membrane, respectively (17, 29, 30). Although GPBP does not contain the structural requirements to be exported, the 5'-end untranslated region of its mRNA includes an upstream ORF of 130 residues with an in-frame stop codon at the beginning (Fig. 1). A mRNA editing process inserting a single base pair (U) would generate an operative in-frame start site and an ORF of 754-residues containing an export signal immediately downstream of the edited Met (not shown). Polyclonal antibodies against a synthetic peptide representing part of this hypothetical extra-sequence (PR SAR CQARRRRGGRTSS (SEQ ID NO:33)) display a linear vascular reactivity in human tissues suggestive of an extracellular basement membrane localization (data not shown).

[0084] Alternatively, a splicing phenomenon could generate transcripts with additional unidentified exon(s) that would provide the structural requirements for exportation. The multiple cellular localization, the high content in PTyr, and the lack of tyrosine kinase activity *in vitro*, suggest that GPBP is itself the target of specific tyrosine kinase(s) and therefore likely involved in specific signaling cascade(s).

[0085] As discussed above, specific serine phosphorylation, as well as pre-mRNA alternative splicing, are associated with the biology of several autoantigens, including the GP antigen, acetylcholine receptor and myelin basic protein (MBP) (4). The latter is suspected to be the major antigen in multiple sclerosis (MS), another exclusively human autoimmune disease in which the immune system targets the white matter of the central nervous system. GP disease and MS are human disorders that display a strong association with the same HLA class II haplotype (HLA DRB1*1501)(32, 33). This, along with the recent report of death by GP disease of a MS patient carrying this HLA specificity (34), supports the existence of common pathogenic events in these human disorders.

[0086] Phosphorylation of specific serines has been shown to change intracellular proteolysis (35-40). Conceivably, alterations in protein phosphorylation can affect processing and peptide presentation, and thus mediate autoimmunity. GP antigen-derived peptide presentation by the HLA-DR15 depends more on processing than on preferences of relatively indiscriminate DR15 molecules (41), suggesting that if processing is influenced by abnormal phosphorylation, the resulting peptides would likely be presented by this HLA. Our more recent data indicate that in both the GP and MBP systems, the production of alternative splicing products serves to regulate the phosphorylation of specific and structurally homologous PKA sites, suggesting that this or a closely related kinase is the *in vivo* phosphorylating enzyme. Alterations in the degree of antigen phosphorylation, caused either by an imbalance in alternative products, or by the action of an intruding kinase that deregulates phosphorylation of the same motifs, could lead to an autoimmune response in predisposed individuals. rGPBP phosphorylates the human GP antigen at a major PKA phosphorylation site in an apparently unregulated fashion, since the presence of specific alternative products of the GP antigen did not affect phosphorylation of the primary antigen by GPBP (not shown).

[0087] Although GPBP is ubiquitously expressed, in certain organs and tissues it shows a preference for cells and tissue structures that are target of common autoimmune responses: the Langerhans cells (type I diabetes); the white matter of the central nervous system (multiple sclerosis); the biliary ducts (primary biliary cirrhosis); the cortical cells of the adrenal gland (Addison disease); striated muscle cells (myasthenia gravis); spermatogonium (male infertility); Purkinje cells of the cerebellum (paraneoplastic cerebellar degeneration syndrome); and intestinal epithelial cells (pernicious anemia, autoimmune gastritis and enteritis). All the above observations point to this novel kinase as an attractive candidate to be considered when envisioning a model for human autoimmune disease.

References for the Background and Example 1

[0088]

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Example 2: GPBP Alternative Splicing

5 **[0089]** Here we report the existence of two isoforms of GPBP that are generated by alternative splicing of a 78-base pair (bp) long exon that encodes a 26-residue serine-rich motif. Both isoforms, GPBP and GPBP Δ 26, exist as high molecular aggregates that result from polypeptide self-aggregation. The presence of the 26-residue peptide in the polypeptide chain results in a molecular species that self-interacts more efficiently and forms aggregates with higher specific activity. Finally, we present evidences supporting the observation that GPBP is implicated in human autoimmune pathogenesis.

MATERIAL AND METHODS.**Synthetic polymers:**

15 **[0090] Peptides.** GPpep1, KGKRGDSGSPATWTTRGFVFT (SEQ ID NO:26), is described in Example 1. GPBPpep1, PYSRSSSMSSIDLVSASDDVHRFSSQ (SEQ ID NO:14), representing residues 371-396 of GPBP was synthesized by Genosys.

20 **Oligonucleotides.** The following oligonucleotides were synthesized by Life Technologies, Inc., 5' to 3': ON-GPBP-11m, G CGG GAC TCA GCG GCC GGA TTT TCT (SEQ ID NO:34); ON-GPBP-15m, AC AGC TGG CAG AAG AGA C (SEQ ID NO:35); ON-GPBP-20c, C ATG GGT AGC TTT TAA AG (SEQ ID NO: 36); ON-GPBP-22m, TA GAA GAA CAG TCA CAG AGT GAA AAG G (SEQ ID NO:37); ON-GPBP-53c, GAATTC GAA CAA AAT AGG CTT TC (SEQ ID NO:38); ON-GPBP-56m, CCC TAT AGT CGC TCT TC (SEQ ID NO:39); ON-GPBP-57c, CTG GGA GCT GAA TCT GT (SEQ ID NO:40); ON-GPBP-62c, GTG GTT CTG CAC CAT CTC TTC AAC (SEQ ID NO:41); ON-GPBP- Δ 26, CA CAT AGA TTT GTC CAA AAG GTT GAA GAG ATG GTG CAG AAC (SEQ ID NO:42).

25 **Reverse transcriptase and polymerase chain rection (RT-PCR).** Total RNA was prepared from different control and GP tissues as described in (15). Five micrograms of total RNA was retrotranscribed using Ready-To-Go You-Prime First-Strand beads (Amersham Pharmacia Biotech) and 40 pmol of ON-GPBP-53c. The corresponding cDNA was subjected to PCR using the pairs of primers ON-GPBP-11m/ON-GPBP-53c or ON-GPBP-15m/ON-GPBP-62c. The identity of the products obtained with 15m-62c was further confirmed by Alu I restriction. To specifically amplify GPBP transcripts, PCR was performed using primers ON-GPBP-15m/ON-GPBP-57c.

30 **Northern hybridization studies.** Pre-made human multiple-tissue and tumor cell-line Northern Blots (CLONTECH) were probed with a cDNA containing the 78-bp exon present only in GPBP or with a cDNA representing both isoforms. The corresponding cDNAs were obtained by PCR using the pair of primers ON-GPBP-56m and ON-GPBP-57c using GPBP as a template, or with primers ON-GPBP-22m and ON-GPBP-20c, using GPBP Δ 26 as a template. The resulting products were random-labeled and hybridized following the manufacturers' instructions.

35 **Plasmid construction, expression and purification of recombinant proteins.** The plasmid pHIL-FLAG-n4', used for recombinant expression of FLAG-tagged GPBP in *Pichia pastoris* has been described elsewhere (4). The sequence coding for the 78-bp exon was deleted by site-directed mutagenesis using ON-GPBP- Δ 26 to generate the plasmid pHIL-FLAG-n4' Δ 26. Expression and affinity-purification of recombinant GPBP and GPBP Δ 26 was done as in (4).

40 **Gel-filtration HPLC.** Samples of 250 μ l were injected into a gel filtration PE-TSK-G4000SW HPLC column equilibrated with 50 mM Tris-HCl pH 7.5, 150 mM NaCl. The material was eluted from the column at 0.5 ml/min, monitored at 220 nm and minute fractions collected.

45 **In vitro phosphorylation assays.** The auto-, trans-phosphorylation and in-blot renaturation studies were performed as in Example 1.

50 **Antibodies and immunochemical techniques.** Polyclonal antibodies were raised by in chicken against a synthetic peptide (GPBPpep1) representing the sequence coded by the 78-bp exon (Genosys). Egg yolks were diluted 1:10 in water, the pH adjusted to 5.0. After 6 hours at 4°C, the solution was clarified by centrifugation (25 min at 10000 x g at 4°C) and the antibodies precipitated by adding 20 % (w/v) of sodium sulfate at 20.000 x g, 20'. The pellets were dissolved in PBS (1 ml per yolk) and used for immunohistochemical studies. The production of antibodies against GPBP/GPBP Δ 26 or against α 3(IV)NC1 domain are discussed above (see also 4, 13).

55 **Sedimentation velocity.** Determination of sedimentation velocities were performed in an Optima XL-A analytical ultracentrifuge (Beckman Instruments Inc.), equipped with a VIS-UV scanner, using a Ti60 rotor and double sector cells of Epon-charcoal of 12 mm optical path-length. Samples of ca. 400 μ l were centrifuged at 30,000 rpm at 20°C and radial scans at 220 nm were taken every 5 min. The sedimentation coefficients were obtained from the rate of movement of the solute boundary using the program XLAVEL (supplied by Beckman).

Sedimentation equilibrium. Sedimentation equilibrium experiments were done as described above for velocity experiments with samples of 70 μ l, and centrifuged at 8,000 rpm. The experimental concentration gradients at equilibrium

were analyzed using the program EQASSOC (Beckman) to determine the corresponding weight average molecular mass. A partial specific volumes of 0.711 cm³/g for GPBP and 0.729 cm³/g for GPBP Δ 26 were calculated from the corresponding amino acid compositions.

Physical methods and immunochemical techniques. SDS-PAGE and Western blotting were performed under reducing conditions as previously described (3). Immunohistochemistry studies were done on formalin fixed paraffin embedded tissues using the ABC peroxidase method (4) or on frozen human biopsies fixed with cold acetone using standard procedures for indirect immunofluorescence.

Two hybrid studies. Self-interaction studies were carried out in *Saccharomyces cerevisiae* (HF7c) using pGBT9 and pGAD424 (CLONTECH) to generate GAL4 binding and activation domain-fusion proteins, respectively. Interaction was assessed following the manufacturer's recommendations. β -galactosidase activity was assayed with X-GAL (0.75 mg/ml) for *in situ* and with ortho-nitrophenyl β -D galactopyranoside (0.64 mg/ml) for the in-solution determinations.

RESULTS

[0091] Identification of two spliced GPBP variants. To characterize the GPBP species in normal human tissues, we coupled reverse transcription to a polymerase chain reaction (RT-PCR) on total RNA from different tissues, using specific oligonucleotides that flank the full open reading frame of GPBP. A single cDNA fragment displaying lower size than expected was obtained from skeletal muscle-derived RNA (Fig. 8A), and from kidney, lung, skin, or adrenal gland-derived RNA (not shown). By combining nested PCR re-amplifications and endonuclease restriction mapping, we determined that all the RT-PCR products corresponded to the same molecular species (not shown). We fully sequenced the 2.2-Kb of cDNA from human muscle and found it identical to HeLa-derived material except for the absence of 78-nucleotides (positions 1519-1596), which encode a 26-residues motif (amino acids 371-396) (Fig. 8B). We therefore named this more common isoform of GPBP as GPBP Δ 26.

[0092] To investigate whether the 78-bp represent an exon skipped transcript during pre-mRNA processing, we used this cDNA fragment to probe a human-derived genomic library and we isolated a ~14-Kb clone. By combining Southern blot hybridization and PCR, the genomic clone was characterized and a contiguous DNA fragment of 12482-bp was fully sequenced (SEQ ID 25). The sequence contained (from 5' to 3'), 767-bp of intron sequence, a 93-bp exon, an 818-bp intron, the 78-bp exon sequence of interest, a 9650-bp intron, a 96-bp exon and a 980-bp intron sequence (Fig. 8C). The exon-intron boundaries determined by comparing the corresponding DNA and cDNA sequences meet the canonical consensus for 5' and 3' splice sites (Fig 8C) (5), thus confirming the exon nature of the 78-bp sequence. The GPBP gene was localized to chromosome 5q13 by fluorescence *in situ* hybridization (FISH) using the genomic clone as a probe (not shown).

[0093] The relative expression of GPBP in human-derived specimens was assessed by Northern blot analysis, using either the 78-bp exon or a 260-bp cDNA representing the flanking sequence of 78-bp (103-bp 5' and 157-bp 3') present in both GPBP and GPBP Δ 26 (Fig. 9). The 78-bp containing the molecular species of interest were preferably expressed in striated muscle (both skeletal and heart) and brain, and poorly expressed in placenta, lung and liver. In contrast to GPBP Δ 26, the GPBP was expressed at very low levels in kidney, pancreas and cancer cell lines.

[0094] All the above indicates that GPBP is expressed at low levels in normal human tissues, and that the initial lack of detection by RT-PCR of GPBP can be attributed to a preferential amplification of the more abundant GPBP Δ 26. Indeed, the cDNA of GPBP could be amplified from human tissues (skeletal muscle, lung, kidney, skin and adrenal gland) when the specific RT-PCR amplifications were done using 78-bp exon-specific oligonucleotides (not shown). This also suggests that GPBP Δ 26 mRNA is the major transcript detected in Northern blot studies when using the cDNA probe representing both GPBP and GPBP Δ 26.

[0095] Recombinant expression and functional characterization of GPBP Δ 26. To investigate whether the absence of the 26-residue serine-rich motif would affect the biochemical properties of GPBP, we expressed and purified both isoforms (rGPBP and rGPBP Δ 26), and assessed their auto- and trans-phosphorylation activities (Fig. 10). As reported above for rGPBP (see also 4), rGPBP Δ 26 is purified as a single major polypeptide and several related minor products (Fig. 10 A). However, the number and relative amounts of the derived products vary compared to rGPBP, and they display M_r on SDS-PAGE that cannot be attributed simply to the 26-residue deletion. This suggests that the 26-residue motif has important structural and functional consequences that could account for the reduced in-solution auto- and trans-phosphorylation activities displayed by rGPBP Δ 26 (Fig. 10B). Interestingly, the differences in specific activity shown in the in-solution assays were not evident when autophosphorylation was assessed in-blot after SDS-PAGE and renaturation, suggesting that the 26-residue motif likely has important functional consequences at the quaternary structure level. Renaturation studies further showed that phosphate transfer activities reside in the major polypeptides representing the proposed open reading frames, and are not detectable in derived minor products.

[0096] rGPBP and rGPBP-26 exist as very active high molecular weight aggregates. Gel filtration analysis of affinity-purified rGPBP or rGPBP Δ 26 yielded two chromatographic peaks (I and II), both displaying higher MW than expected for the individual molecular species, as determined by SDS-PAGE studies (89 kDa and 84 kDa, respectively)

(Fig. 11). The bulk of the recombinant material eluted as a single peak between the 158 kDa and the 669 kDa molecular weight markers (peak II), while limited amounts of rGPBP and only traces of rGPBP Δ 26 eluted in peak I (>1000 kDa). Aliquots of fractions representing each chromatographic profile were subjected to SDS-PAGE and stained, or incubated in the presence of ^{32}P [γ] ATP, and analyzed by immunoblot and autoradiography. Along with the major primary polypeptide, every chromatographic peak contained multiple derived products of higher or lower sizes indicating that the primary polypeptide associates to form high molecular weight aggregates that are stabilized by covalent and non-covalent bonds (not shown). The kinase activity also exhibited two peaks coinciding with the chromatographic profiles. However, peak I showed a much higher specific activity than peak II, indicating that these high molecular weight aggregates contained a much more active form of the kinase. Equal volumes of rGPBP fractions number 13 and 20 exhibited comparable phosphorylating activity, even though the protein content is approximately 20 times lower in fraction 13, as estimated by Western blot and Coomassie blue staining (Fig. 11A). The specific activities of rGPBP and rGPBP Δ 26 at peak II are also different, and are consistent with the studies shown for the whole material, thus supporting the hypothesis that the presence of the 26-residue serine-rich motif renders a more active kinase. These results also suggest that both rGPBP and rGPBP Δ 26 exist as oligomers under native conditions, and that both high molecular weight aggregate formation and specific activity are greatly dependent on the presence of the 26-residue serine-rich motif. Analytical centrifugation analysis of rGPBP revealed that peak I contained large aggregates (over 10^7 Da). Peak II of rGPBP contained a homogeneous population of 220 ± 10 kDa aggregates, likely representing trimers with a sedimentation coefficient of 11S. Peak II of rGPBP Δ 26 however consisted of a more heterogeneous population that likely contains several oligomeric species. The main population (ca. 80%) displayed a weight average molecular mass of 310 ± 10 kDa and a coefficient of sedimentation of 14S.

[0097] GPBP and GPBP Δ 26 self-interact in a yeast two-hybrid system. To assess the physiological relevance of the self-aggregation, and to determine the role of the 26-residue motif, we performed comparative studies using a two-hybrid interaction system in yeast. In this type of study, the polypeptides whose interaction is under study are expressed as a part of a fusion protein containing either the activation or the binding domains of the transcriptional factor GAL4. An effective interaction between the two fusion proteins through the polypeptide under study would result in the reconstitution of the transcriptional activator and the subsequent expression of the two reporter genes, Lac Z and His3, allowing colony color detection and growth in a His-defective medium, respectively. We estimated the intensity of interactions by the growth-rate in histidine-defective medium, in the presence of different concentrations of a competitive inhibitor of the His3 gene product (3-AT), and a quantitative colorimetric liquid β -galactosidase assay. A representative experiment is presented in Fig. 12. When assaying GPBP Δ 26 for self-interaction, a significant induction of the reporter genes was observed, while no expression was detectable when each fusion protein was expressed alone or with control fusion proteins. The insertion of the 26-residue motif in the polypeptide to obtain GPBP resulted in a notable increase in polypeptide interaction. All of the above data indicate that GPBP Δ 26 self-associates *in vivo*, and that the insertion of the 26-residues into the polypeptide chain yields a more interactive molecular species.

[0098] GPBP is highly expressed in human but not in bovine and murine glomerulus and alveolus. We have shown that GPBP/GPBP Δ 26 is preferentially expressed in human cells and tissues that are commonly targeted in naturally occurring autoimmune responses. To specifically investigate the expression of GPBP, we raised polyclonal antibodies against a synthetic peptide representing the 26-residue motif characteristic of this kinase isoform, and used it for immunohistochemical studies on frozen or formalin fixed paraffin embedded human tissues (Fig 13). In general, these antibodies showed more specificity than the antibodies recognizing both isoforms for the tissue structures that are target of autoimmune responses such as the biliary ducts, the Langerhans islets or the white matter of the central nervous system (not shown). Nevertheless, the most remarkable finding was the presence of linear deposits of GPBP-selective antibodies around the small vessels in every tissue studied (A), suggesting that GPBP is associated with endothelial basement membranes. Consequently, at the glomerulus, the anti-GPBP antibodies displayed a vascular pattern closely resembling the glomerular basement membrane staining yielded either by monoclonal antibodies specifically recognizing the α 3(IV)NC1 (compare 13B with 13C and 13D), or by circulating GP autoantibodies (compare 13E and 13F). These observations further supported the initial observation that GPBP is expressed in tissue structures targeted in natural autoimmune responses, suggesting that the expression of GPBP is a risk factor and makes the host tissue vulnerable to an autoimmune attack.

[0099] To further assess this hypothesis, we investigated the presence of GPBP and GPBP Δ 26 in the glomerulus of two mammals that naturally do not undergo GP disease compared to human (Fig.14). GPBP-specific antibodies failed to stain the glomerulus of both bovine or murine specimens (compare 14A with 14B and 14C) while antibodies recognizing the N-terminal sequence common to both GPBP and GPBP Δ 26 stained these structures in all three species, although with different distributions and intensities (14D-14F). In bovine renal cortex, GPBP Δ 26 was expressed at a lower rate than in human, but showed similar tissue distribution. In murine samples, however, GPBP Δ 26 displayed a tissue distribution closely resembling that of GPBP in human glomerulus. Similar results were obtained when studying the alveolus in the three different species (not shown). To rule out that the differences in antibody detection was due to primary structure differences rather than to a differential expression, we determined the corresponding primary structures in

these two species by cDNA sequencing. Bovine and mouse GPBP (SEQ ID NOS:3-6 and 9-12) displayed an overall identity with human material of 97.9% and 96.6% respectively. Furthermore, the mouse 26-residue motif was identical to human while bovine diverged only in one residue. Finally, and similarly to human, we successfully amplified GPBP cDNA from mouse or bovine kidney total RNA using oligonucleotides specific for the corresponding 78-bp exons, indicating that GPBP is expressed at very low levels not detectable by immunochemical techniques.

[0100] GPBP is highly expressed in several autoimmune conditions. We analyzed several tissues from different GP patients by specific RT-PCR to assess GPBP/GPBP Δ 26 mRNA levels. As in control kidneys, the major expressed isoform in GP kidneys was GPBP Δ 26. However, in the muscle of one of the patients, GPBP was preferentially expressed, whereas GPBP Δ 26 was the only isoform detected in control muscle samples (Fig. 15 A). Since we did not have kidney samples from this particular patient, we could not assess GPBP/GPBP Δ 26 expression in the corresponding target organ. For similar reasons, we could not assess GPBP/GPBP Δ 26 levels in the muscle of the patients in which kidneys were studied. Muscle cells express high levels of GPBP/GPBP Δ 26 (see Northern blot in Fig. 9), and they comprise the bulk of the tissue. In contrast, the expression of GPBP/GPBP Δ 26 in the kidney was much less, and the glomerulus was virtually the only kidney structure expressing the GPBP isoform (see Fig. 13). The glomerulus is a relatively less abundant structure in kidney than the myocyte is in muscle, and the glomerulus is the structure targeted by immune attack in GP pathogenesis. These factors, together with the preferential amplification of the more abundant and shorter messages when performing RT-PCR studies, could account for the lack of detection of GPBP in both normal and GP kidneys, thus precluding the assessment of GPBP expression at the glomerulus during pathogenesis. Nevertheless, the increased levels of GPBP in a GP patient suggest that GPBP/GPBP Δ 26 expression is altered during GP pathogenesis, and that augmented GPBP expression has a pathogenic significance in GP disease.

[0101] To investigate the expression of GPBP and GPBP Δ 26 in autoimmune pathogenesis, we studied cutaneous autoimmune processes and compared them with control samples representing normal skin or non-autoimmune dermatitis (Fig. 15). Control samples displayed a limited expression of GPBP in the most peripheral keratinocytes (15B, 15E), while keratinocytes expanding from stratum basale to corneum expressed abundant GPBP in skin affected by systemic lupus erythematosus (SLE) (15C, 15F) or lichen planus (15D, 15G). GPBP was preferentially expressed in cell surface structures that closely resembled the blebs previously described in cultured keratinocytes upon UV irradiation and apoptosis induction (6). In contrast, antibodies recognizing both GPBP and GPBP Δ 26 yielded a diffuse cytosolic pattern through the whole epidermis in both autoimmune affected or control samples (not shown). These data indicate that in both control and autoimmune-affected keratinocytes, GPBP Δ 26 was expressed at the cytosol and that the expression did not significantly vary during cell differentiation. In contrast, mature keratinocytes were virtually the only GPBP expressing cells. However, bleb formation and expression of GPBP was observed in the early stages of differentiation in epidermis affected by autoimmune responses (15C, 15D, 15F, 15G). This further supports previous observations indicating that aberrant apoptosis at the basal keratinocytes is involved in the pathogenesis of autoimmune processes affecting skin (7), and suggests that apoptosis and GPBP expression are linked in this human cell system.

DISCUSSION

[0102] Alternative pre-mRNA splicing is a fundamental mechanism for differential gene expression that has been reported to regulate the tissue distribution, intracellular localization, and function of different protein kinases (8-11). In this regard, and closely resembling GPBP, B-Raf exists as multiple spliced variants, in which the presence of specific exons renders more interactive, efficient and oncogenic kinases (12).

[0103] Although it is evident that rGPBP Δ 26 still bears the uncharacterized catalytic domain of this novel kinase, both auto- and trans-phosphorylating activities are greatly reduced when compared to rGPBP. Gel filtration and two hybrid experiments provide some insights into the mechanisms that underlie such a reduced phosphate transfer activity. About 1-2% of rGPBP is organized in very high molecular weight aggregates that display about one third of the phosphorylating activity of rGPBP, indicating that high molecular aggregation renders more efficient quaternary structures. Recombinant GPBP Δ 26, with virtually no peak I material, consistently displayed a reduced kinase activity. However, aggregation does not seem to be the only mechanism by which the 26-residues increases specific activity, since the rGPBP Δ 26 material present in peak II also shows a reduced phosphorylating activity when compared to homologous fractions of rGPBP. One possibility is that rGPBP-derived aggregates display higher specific activities because of quaternary structure strengthening caused by the insertion of the 26-residue motif. The oligomers are kept together mainly by very strong non-covalent bonds, since the bulk of the material appears as a single polypeptide in non-reducing SDS-PAGE, and the presence of either 8 M urea or 6 M guanidine had little effect on chromatographic gel filtration profiles (not shown). How the 26-residue motif renders a more strengthened and active structure remains to be clarified. Conformational changes induced by the presence of an exon encoded motif that alter the activation status of the kinase have been proposed for the linker domain of the Src protein (24) and exons 8b and 10 of B-Raf (12). Alternatively, the 26-residue motif may provide the structural requirements such as residues whose phosphorylation may be necessary for full activation of GPBP.

[0104] We have reported (13) that the primary structure of the GP antigen (α 3(IV)NC1) is the target of a complex

folding process yielding multiple conformers. Isolated conformers are non-minimum energy structures specifically activated by phosphorylation for supramolecular aggregation and likely quaternary structure formation. In GP patients, the $\alpha 3(\text{IV})\text{NC1}$ shows conformational alterations and a reduced ability to mediate the disulfide stabilization of the collagen IV network. The GP antibodies, in turn, demonstrate stronger affinity towards the patient $\alpha 3(\text{IV})\text{NC1}$ conformers, indicating that conformationally altered material caused the autoimmune response. Therefore, it seems that in GP disease an early alteration in the conforming process of the $\alpha 3(\text{IV})\text{NC1}$ could generate altered conformers for which the immune system is not tolerant, thus mediating the autoimmune response.

[0105] Other evidence (Raya et al., unpublished results) indicates that phosphorylation is the signal that drives the folding of the $\alpha 3(\text{IV})\text{NC1}$ into non-minimum energy ends. In this scenario, three features of the human $\alpha 3(\text{IV})\text{NC1}$ system are of special pathogenic relevance when compared to the corresponding antigen systems from species that, like bovine or murine, do not undergo spontaneous GP disease. First, the N-terminus of the human $\alpha 3(\text{IV})\text{NC1}$ contains a motif that is phosphorylatable by PKA and also by GPBP (see above, and also 2-4). Second, the human gene generates multiples alternative products by alternative exon splicing (14,15). Exon skipping generates alternative products with divergent C-terminal ends that up-regulate the *in vitro* PKA phosphorylation of the primary $\alpha 3(\text{IV})\text{NC1}$ product (See below Example 3). Third, the human GPBP is expressed associated with glomerular and alveolar basement membranes, the two main targets in GP disease. The phosphorylation-dependent conforming process is also a feature of non-pathogenic NC1 domains (13), suggesting that the phosphorylatable N-terminus, the alternative splicing diversification, and the expression of GPBP at the glomerular and alveolar basement membranes, are all exclusively human features that place the conformation process of $\alpha 3(\text{IV})\text{NC1}$ in a vulnerable condition. The four independent GP kidneys studied expressed higher levels of GP antigen alternative products (15; Bernal and Saus, unpublished results), and an augmented expression of GPBP were found in a GP patient (see above). Both increased levels of alternative GP antigen products and GPBP are expected to have consequences in the phosphorylation-dependent conformational process of the $\alpha 3(\text{IV})\text{NC1}$, and therefore with pathogenic potential.

[0106] GPBP is highly expressed in skin targeted by natural autoimmune responses. In the epidermis, GPBP is associated with cell surface blebs characteristic of the apoptosis-mediated differentiation process that keratinocytes undergo during maturation from basale to corneum strata (22, 23). Keratinocytes from SLE patients show a remarkably heightened sensitivity to UV-induced apoptosis (6, 18, 20), and augmented and premature apoptosis of keratinocytes has been reported to exist in SLE and dermatomyositis (7). Consistently, we found apoptotic bodies expanding from basal to peripheral strata of the epidermis in several skin autoimmune conditions including discoid lupus (not shown), SLE and lichen planus. Autoantigens, and modified versions thereof are clustered in the cell surface blebs of apoptotic keratinocytes (6,18,20). Apoptotic surface blebs present autoantigens (21), and likely release modified versions to the circulation (16-20). It has been suggested that the release of modified autoantigens from apoptotic bodies could be the immunizing event that mediates systemic autoimmune responses mediating SLE and scleroderma (18,19).

[0107] Our evidence indicates that both GPBP and GPBP $\Delta 26$ are able to act *in vitro* as protein kinases, with GPBP being a more active isoform than GPBP $\Delta 26$. Furthermore, recombinant material representing GPBP or GPBP $\Delta 26$ purified from yeast or from human 293 cells contained an associated proteolytic activity that specifically degrades the $\alpha 3(\text{IV})\text{NC1}$ domain (unpublished results). The proteolytic activity operates on $\alpha 3(\text{IV})\text{NC1}$ produced in an eukaryotic expression system, but not on recombinant material produced in bacteria (unpublished results), indicating that $\alpha 3(\text{IV})\text{NC1}$ processing has some conformational or post-translational requirements not present in prokaryotic recombinant material. Finally, it has been reported that several autoantigens undergo phosphorylation and degradation in apoptotic keratinocytes (20). While not being limited to an exact mechanism, we propose, in light of all of the above data, that the machinery assembling GPBP at the apoptotic blebs likely performs a complex modification of the autoantigens that includes phosphorylation, conformational changes and degradation. Accordingly, recombinant protein representing autoantigens in SLE (P1 ribosomal phosphoprotein and Sm-D1 small nuclear ribonucleoproteins) and in dermatomyositis (hystidil-tRNA synthetase) were *in vitro* substrates of GPBP (unpublished results).

[0108] The down-regulation in cancer cell lines of GPBP, suggest that the cell machinery harboring GPBP/GPBP $\Delta 26$ is likely involved in signaling pathways inducing programmed cell death. The corresponding apoptotic pathway could be up regulated during autoimmune pathogenesis to cause an altered antigen presentation in individuals carrying specific MHC haplotypes; and down regulated during cell transformation to prevent autoimmune attack to the transformed cells during tumor growth.

References for Example 2

[0109]

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Example 3. Regulation of Human Autoantigen Phosphorylation by Exon Splicing

30 INTRODUCTION

[0110] In GP disease, the immune system attack is mediated by autoantibodies against the non-collagenous C-terminal domain (NC1) of the $\alpha 3$ chain of collagen IV (the GP antigen) (1). The N-terminus of the human $\alpha 3$ (IV)NC1 contains a highly divergent and hydrophilic region with a unique structural motif, KRGDS⁹, that harbors a cell adhesion signal as an integral part of a functional phosphorylation site for type A protein kinases (2,3). Furthermore, the gene region encoding the human GP antigen characteristically generates multiple mRNAs by alternative exon splicing (4,5). The alternative products diverge in the C-terminal ends and all but one share the N-terminal KRGDS⁹ (4,5).

[0111] Multiple sclerosis (MS) is an exclusive human neurological disease characterized by the presence of inflammatory demyelization plaques at the central nervous system. (6). Several evidences indicate that this disease is caused by an autoimmune attack mediated by cytotoxic T cells towards specific components of the white matter including the myelin basic protein (MBP) (7, 8). In humans, the MBP gene generates four products (MBP, MBP Δ II, MBP Δ V and MBP Δ II/V) that result from alternative exon splicing during pre-mRNA processing (9). Among these, MBP Δ II is the more abundant form in the mature central nervous system, while MBP form containing all the exons is virtually absent (9).

[0112] Several biological similarities exist between the autoimmune responses mediating GP disease and MS, namely: 1) both are human exclusive diseases and typically initiate after a viral flu-like disease; 2) a strong linkage exists to the same haplotype of the HLA-DR region of the class II MHC; 3) several products are generated by alternative splicing; and 4) the death of a MS patient by GP disease has recently been reported (10).

50 MATERIALS AND METHODS

[0113] **Synthetic polymers:** GP Δ III derived peptide, QRAHGQDLDFVVKVLRSP (SEQ ID NO:43) and GP Δ III/IV/V derived peptide, QRAHGQDLESLFHQL (SEQ ID NO:44) were synthesized using either Boc- (MedProbe) or Fmoc- (Chiron, Lipotec) chemistry.

55 Plasmid construction and recombinant expression.

[0114] **GP derived material:** The constructs representing the different GP-spliced forms were obtained by subcloning the cDNAs used elsewhere to express the corresponding recombinant proteins (5) into the BamHI site of a modified

pET15b vector, in which the extraneous vector-derived amino-terminal sequence except for the initiation Met was eliminated. The extra sequence was removed by cutting the vector with NcoI and Bam HI, filling-in of the free ends with Klenow, and re-ligation. This resulted in the reformation of both restriction sites and placed the BamHI site immediately downstream of the codon for the amino-terminal Met.

5 **[0115]** The recombinant proteins representing GP or GP Δ V (SEQ ID NO:46) were purified by precipitation (5). Bacterial pellets containing the recombinant proteins representing GP Δ III (SEQ ID NO:48) or GP Δ III/IV/V (SEQ ID NO:50) were dissolved by 8 M urea in 40 mM Tris-HCl pH 6.8 and sonication. After centrifugation at 40,000 x g the supernatants were passed through a 0.22 μ m filter and applied to resource Q column for FPLC. The effluent was acidified to pH 6 with HCl and applied to a resource S column previously equilibrated with 40 mM MES pH 6 for a second FPLC purification. The material in the resulting effluent was used for *in vitro* phosphorylation.

10 **[0116] MBP-derived material:** cDNA representing human MBP Δ II (SEQ ID NO:51) was obtained by RT-PCR using total RNA from central nervous system. The cDNA representing human MBP was a generous gift from C. Campagnoni (UCLA). Both fragments were cloned into a modified version of pHIL-D2 (Invitrogen) containing a 6xHis-coding sequence at the C-terminus to generate pHIL-MBP Δ II-His and pHIL-MBP-His, respectively. These plasmids were used for recombinant expression in *Pichia pastoris* as described in (12). Recombinant proteins were purified using immobilized metal affinity chromatography (TALON resin, CLONTECH) under denaturant conditions (8M urea) and eluted with 300 mM imidazole following manufacturers' instructions. The affinity-purified material was then renatured by dilution into 80 volumes of 50 mM Tris-HCl pH 8.0, 10 mM CHAPS, 400 mM NaCl, 2 mM DTT, and concentrated 50 times by ultrafiltration through a YM10-type membrane (AMICON). The Ser to Ala mutants were produced by site-directed mutagenesis over native sequence-containing constructs using transformer mutagenesis kit from CLONTECH and the resulting proteins were similarly produced.

15 **[0117] Phosphorylation studies.** Phosphorylation studies were essentially done as described above (see also 3 and 12). In some experiments, the substrates were in-blot renatured and then, phosphorylated for 30 min at room temperature by overlaying 100 μ l of phosphorylation buffer containing 0.5 μ g of rGPBP. Digestion with V8 endopeptidase and immunoprecipitation were performed as described in (3).

20 **[0118] Antibody production.** Synthetic peptides representing the C-terminal divergent ends of GP Δ III or GP Δ III/IV/V comprised in SEQ ID NO:43 or SEQ ID NO:44 respectively were conjugated to a cytochrome C, BSA or ovalbumine using a glutaraldehyde coupling standard procedure. The resulting protein conjugates were used for mouse immunization to obtain polyclonal antibodies specific for GP Δ III and monoclonal antibodies specific for GP Δ III/IV/V (Mab153). To obtain monoclonal antibodies specific for GP Δ V (Mab5A) mouse were immunized using recombinant bacterial protein representing the corresponding alternative form comprising the SEQ ID NO:50. The production of monoclonal (M3/1, P1/2) or polyclonal (anti-GPpep1) antibodies against SEQ ID NO: 26 which represents the N-terminal region of the GP alternative forms have been previously described (3,5).

35 **Boc-based peptide synthesis.**

[0119] Assembling. The peptide was assembled by stepwise solid phase synthesis using a Boc-Benzyl strategy. The starting resin used was Boc-Pro-PAM resin (0.56 meq/g, batch R4108). The deprotection /coupling procedure used was: TFA (1x1min) TFA (1x 3 min) DCM (flow flash) Isopropylalcohol (1x 30 sec) DMF (3 x 1 min) COUPLING/DMF (1 x10 min) DMF (1x1 min) COUPLING/DMF (1x 10 min) DMF (2x 1min) DCM (1x 1min). For each step 10 ml per gram of peptide-resin were used. The coupling of all amino acids (fivefold excess) was performed in DMF in the presence of BOP, Hobt and DIEA. For the synthesis the following side-chain protecting groups were used: benzyl for serine; 2 chlorobenzoyloxycarbonyl for lysine; cyclohexyl for aspartic and glutamic acid; tosyl for histidine and arginine.

40 **[0120] Cleavage.** The peptide was cleaved from the resin and fully deprotected by a treatment with liquid Hydrogen Fluoride (HF): Ten milliliters of HF per gram of peptide resin were added and the mixture kept at 0° C for 45 min in the presence of p-cresol as scavengers. After evaporation of the HF, the crude reaction mixture is washed with ether, dissolved in TFA, precipitated with ether and dried.

45 **[0121] Purification.** Stationary phase: Silica C18, 15 μ m, 120 A; Mobile phase: solvent A: water 0.1% TFA and solvent B: acetonitrile 1A, 60/40 (v/v); Gradient: linear from 20 to 60% B in 30 min; Flow rate: 40 ml/min; and detection was U.V (210 nm). Fractions with a purity higher than 80% were pooled and lyophilized. Control of purity and identity was performed by analytical HPLC and ES/MS. The final product had 88% purity and an experimental molecular weight of 2192.9.

Fmoc-based peptide synthesis.

55 **[0122] Assembling.** The peptides were synthesized by stepwise linear solid phase on Pro-chlorotriyl-resin (0.685 meq/g) with standard Fmoc/tBu chemistry. The deprotection /coupling procedure used was: Fmoc aa (0.66 g) HOBt (0.26 g) DIPCDI (0.28 ml) for 40 min following a control by Kaiser test. If the test was positive the time was extended until change to negative. Then DMF (31 min), piperidine/DMF 20% (11 min) piperidine/DMF 20% (15 min) and DMF (41 min). Side

chain protectors were: Pmc (pentamethylchromane sulfonyl) for arginine, Bcc (tert-butoxycarbonyl) for lysine, tBu (tert-butyl) for aspartic acid and for serine and TrI (trityl) for histidine.

[0123] Cleavage. The peptide was cleaved and fully deprotected by treatment cleavage with TFA/water 90/10. Ten milliliters of TFA solution per gram of resin were added. Water acts as scavenger. After two hours, resin was filtered and the resulting solution was precipitated five times with cold diethylether. The final precipitated was dried.

[0124] Purification. Stationary phase: Kromasil C 18 10 μm ; Mobile phase: solvent A: water 0.1% TFA and solvent B: acetonitrile 0.1 % TFA; Isocratic: 28% B; Flow rate: 55 ml/min; Detection: 220 nm. Fractions with the higher purity were pooled and lyophilized, and a second HPLC purification round performed. Control of purity and identity was performed by analytical HPLC and ES/MS. The final product had 97% purity and an experimental molecular weight of 2190.9.

RESULTS

[0125] Regulation of the phosphorylation of the human GP antigen by alternative splicing. We produced bacterial recombinant proteins representing the primary antigen (GP) or the individual alternative products GP Δ V (SEQ ID NO:46), GP Δ III (SEQ ID NO:48) and GP Δ III/IV/V (SEQ ID NO:50), and we tested their ability to be phosphorylated by PKA (Figure 16, left panel). Using standard ATP concentrations (150 μM), all four recombinant antigens were phosphorylated but to very different extents. The alternative forms incorporated ^{32}P more efficiently than the primary GP antigen, suggesting that they are better substrates. Because these antigens are expected to be in the extracellular compartment, we also assayed their phosphorylatability with more physiological ATP concentrations (0.1-0.5 μM). Under these conditions, the differences in ^{32}P incorporation between the primary and alternative products were more evident, indicating that at low ATP concentrations the primary GP antigen was a very poor substrate for the kinase. Among the three PKA phosphorylation sites present in the GP antigen, the N-terminal Ser⁹ and Ser²⁶ are the major ones, and are common to all the alternative products assayed (3,5). Accordingly, the differences observed in phosphorylation for the full polypeptides also existed among the individual N-terminal regions, as determined after specific V8 digestion and immunoprecipitation (not shown). This strongly suggests that differences in phosphorylation might be due to the presence of different C-terminal sequences in the alternative products. Since GP Δ III and GP Δ III/IV/V displayed significantly higher ^{32}P incorporation rates than GP Δ V, and they have shorter divergent C-terminal regions (5), we used synthetic peptides individually representing these C-terminal sequences (SEQ ID NO: 43, SEQ ID NO:44) to further examine their regulatory roles in the *in vitro* phosphorylation of the native antigen. Collagen IV is a trimeric molecule comprised of three interwoven α chains. In basement membranes, two collagen IV molecules assemble through their NC1 domains to yield a hexameric NC1 structure that can be solubilized by bacterial collagenase digestion (1). Dissociation of the hexamer structure releases the GP antigen in monomeric and disulfide-related dimeric forms (1). For the following set of experiments, we carried out phosphorylations in the presence of low, extracellular-like ATP concentrations using both monomeric or hexameric native GP antigen (Figure 16, right panel). The presence of each specific peptide but not control peptides (not shown) induced the phosphorylation of a single polypeptide displaying an apparent MW of 22 kDa. By specific V8 digestion and immunoprecipitation, the corresponding polypeptide has been identified as the 22 kDa conformer of the α 3(IV)NC1, previously characterized and identified as the best substrate for the PKA (11).

[0126] Regulation of the phosphorylation of the MBP by alternative splicing. The MBP contains at its N terminal region two PKA phosphorylation sites (Ser⁸, Ser⁵⁷) that are structurally similar to the N terminus site (Ser⁹) present in GP antigen products (Fig 17). The Ser⁸ site present in all the MBP proteins is located in a similar position than the Ser⁹ in the GP-derived polypeptides. In addition, in the MBP and GP Δ III Ser⁸ and Ser⁹ respectively are at a similar distance in the primary structures of a highly homologous motif present in the corresponding exon II (bend arrow in Fig 17). The GP Δ III-derived motif coincides with the C terminal divergent region that up-regulates PKA phosphorylation of Ser⁹ in the GP antigen system (Fig. 16). The regulatory-like sequence in MBP is located at exon II and its presence in the final products depends on an alternative exon splicing mechanism. Therefore, the MBP motif identified by structural comparison to GP Δ III may be also regulating PKA phosphorylation of Ser⁸. We produced recombinant proteins representing MBP and MBP Δ II (SEQ ID NO:54) and the corresponding Ser to Ala mutants to knock-out each of the two PKA phosphorylation sites (Ser⁸ and Ser⁵⁷) present in exon I. Subsequently, we assessed its *in vitro* phosphorylation by PKA (Fig. 18). MBP Δ II was a better substrate than MBP, and Ser⁸ was the major phosphorylation site, indicating that, similarly to GP antigenic system, alternative exon splicing regulates the PKA phosphorylation of specific sites located at the N-terminal region common to all the MBP-derived alternative forms.

[0127] In similar experiments assessing GPBP phosphorylation of the recombinant MBP proteins, GPBP preferentially phosphorylated MBP, while little phosphorylation of MBP Δ II was observed (Fig. 19). Furthermore, recombinant Ser to Ala mutants displayed no significant reduction in ^{32}P incorporation, indicating that GPBP phosphorylates MBP/MBP Δ II in an opposite way than PKA, and that these two kinases do not share major phosphorylation sites in MBP proteins.

[0128] From all these data we concluded that in the MBP system, alternative splicing regulates the phosphorylation of specific serines by either PKA or GPBP.

[0129] Synthetic peptides representing the C terminal region of GP Δ III influence GPBP phosphorylation. To

assess the effect of the C terminal region of GPΔIII on GPBP activity, peptides representing this region were synthesized using two different chemistries (Boc or Fmoc), and separately added to a phosphorylation mixture containing GPBP (Fig. 20). Boc-based synthetic peptides positively influenced GPBP autophosphorylation while Fmoc-based inhibited GPBP autophosphorylation, suggesting that the regulatory sequences derived from the alternative products in either GP and MBP antigenic systems can influence the kinase activity of GPBP.

DISCUSSION

[0130] We have shown that the $\alpha 3(\text{IV})\text{NC1}$ domain undergoes a complex structural diversification by two different mechanism: 1) alternative splicing (4,5) and 2) conformational isomerization of the primary product (11). Both mechanisms generate products that are distinguished by PKA, indicating that PKA phosphorylation is a critical event in the biology of the $\alpha 3(\text{IV})\text{NC1}$ domain. Phosphorylation guides at least in part the folding, but also the supramolecular assembly of the $\alpha 3(\text{IV})\text{NC1}$ domain in the collagen IV network (11 and Raya et al. unpublished results). Altered conformers of the $\alpha 3(\text{IV})\text{NC1}$ lead the autoimmune response mediating GP disease (11), suggesting that an alteration in antigen phosphorylation could be the primary event in the onset of the disease. Accordingly, we have found increased expression levels of GPΔIII in several GP kidneys (4 and Bernal and Saus, unpublished results), and an increased expression of GPBP has been detected in another Goodpasture patient (Fig. 15). Both increased expression of alternative GP antigen products and of GPBP are expected to have consequences in the phosphorylation steady state of $\alpha 3(\text{IV})\text{NC1}$, and therefore in the corresponding conformational process. The discrimination among the different structural products by PKA strongly suggests that this kinase, or another structurally similar kinase, is involved in the physiological antigen conforming process, and that antigen phosphorylation by GPBP has a pathogenic significance. In pathogenesis, GPBP could be an intruding kinase, interfering in the phosphorylation-dependent conforming process. Accordingly, GPBP is expressed in tissue structures that are targeted by natural autoimmune responses, and an increased expression of GPBP is associated with several autoimmune conditions (See examples 1 and 2 above).

[0131] An alternative splicing mechanism also regulates the PKA phosphorylation of specific serines in the MBP antigenic system. MBP is also a substrate for GPBP suggesting that GPBP may play a pathogenic role in multiple sclerosis, and other autoimmune responses.

[0132] All of the above data identify GPBP as a potential target for therapeutics in autoimmune disease. In Fig 20, we show that synthetic peptides representing the C terminal region of GPΔIII (SEQ ID NO:43) modulate the action of GPBP *in vitro*, and therefore we identified this and related sequences as peptide-based compounds to modulate the activity of GPBP *in vivo*. The induction of GP antigen phosphorylation by PKA was achieved when using Boc-based peptides, but not when using similar Fmoc-based peptides. Furthermore, Boc- but not Fmoc-based peptides were *in vitro* substrates of PKA (not shown), indicating that important structural differences exist between both products. Since both products displayed no significant differences in mass spectrometry, one possibility is that the different deprotection procedure used may be responsible for conformational differences in the secondary structure that may be critical for biological activity. Accordingly, Boc-based peptide loses its ability to induce PKA upon long storage at low temperatures.

REFERENCES FOR EXAMPLE 3

[0133]

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| | gtgagtgagt ggatctgggt ctctgccgtt ggettggctc tteccgtett cctcccctcc 120 | | | | | |
| | tccctccctg actgaggttg gcacttaggg ggccgagttc aggtggcggc gccgggcgca 180 | | | | | |
| 35 | gcgcaggggt cacggccacg gcggctgacg gctggaaggg caggctttct tcgccgctcg 240 | | | | | |
| | tcctcctcc cgggtccgct cgggtgcagg cgcgccggcg gcggcgcggc gggcgcgctt 300 | | | | | |
| 40 | cgtccctett cctgttcct cactccccgg agcgggctct cttggcggtg ccatcccccg 360 | | | | | |
| | acccttcacc ccagggacta ggcgcctgca ctggcgcagc tcgccggagcg ggggcccggtc 420 | | | | | |
| | tcctgctcgg ctgtcgcgtc tcc atg tcg gat aac cag agc tgg aac tcg tcg 473 | | | | | |
| 45 | | | Met Ser Asp Asn Gln Ser Trp Asn Ser Ser | | | |
| | | | 1 | 5 | 10 | |
| | ggc tcg gag gag gat ccg gag acg gag tcc ggg ccg cct gtg gag cgc | | | | | 521 |
| | Gly Ser Glu Glu Asp Pro Glu Thr Glu Ser Gly Pro Pro Val Glu Arg | 15 | 20 | 25 | | |
| 50 | tgc ggg gtc ctc agc aag tgg aca aac tat att cat gga tgg cag gat | | | | | 569 |
| | Cys Gly Val Leu Ser Lys Trp Thr Asn Tyr Ile His Gly Trp Gln Asp | 30 | 35 | 40 | | |
| | cgt tgg gta gtt ttg aaa aat aat act ttg agt tac tac aaa tct gaa | | | | | 617 |
| 55 | Arg Trp Val Val Leu Lys Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu | 45 | 50 | 55 | | |

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| | | |
|----|---|------|
| | gat gaa aca gaa tat ggc tgt agg gga tcc atc tgt ctt agc aag gct | 665 |
| | Asp Glu Thr Glu Tyr Gly Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala | |
| | 60 65 70 | |
| 5 | gtg atc acg cct cac gat ttt gat gaa tgc cgg ttt gat atc agt gta | 713 |
| | Val Ile Thr Pro His Asp Phe Asp Glu Cys Arg Phe Asp Ile Ser Val | |
| | 75 80 85 90 | |
| 10 | aat gat agt gtt tgg tac ctt cga gct cag gac ccg gag cac aga cag | 761 |
| | Asn Asp Ser Val Trp Tyr Leu Arg Ala Gln Asp Pro Glu His Arg Gln | |
| | 95 100 105 | |
| 15 | caa tgg gta gac gcc att gaa cag cac aag act gaa tcg gga tat gga | 809 |
| | Gln Trp Val Asp Ala Ile Glu Gln His Lys Thr Glu Ser Gly Tyr Gly | |
| | 110 115 120 | |
| 20 | tct gag tcc agc ttg cgt aga cat ggc tca atg gtg tca ctg gtg tct | 857 |
| | Ser Glu Ser Ser Leu Arg Arg His Gly Ser Met Val Ser Leu Val Ser | |
| | 125 130 135 | |
| 25 | gga gcg agt ggc tat tct gct acg tcc acc tct tct ttc aag aaa ggc | 905 |
| | Gly Ala Ser Gly Tyr Ser Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly | |
| | 140 145 150 | |
| 30 | cac agt tta cgt gag aaa ctg gct gaa atg gag aca ttt cgg gac atc | 953 |
| | His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg Asp Ile | |
| | 155 160 165 170 | |
| 35 | ctg tgc cgg cag gtt gat act ctc cag aag tac ttt gat gtc tgt gct | 1001 |
| | Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe Asp Val Cys Ala | |
| | 175 180 185 | |
| 40 | gac gct gtc tcc aag gat gag ctt cag agg gat aaa gtc gta gaa gat | 1049 |
| | Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val Glu Asp | |
| | 190 195 200 | |
| 45 | gat gaa gat gac ttc cct aca act cgt tct gat gga gac ttt ttg cac | 1097 |
| | Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe Leu His | |
| | 205 210 215 | |
| 50 | aat acc aat ggt aat aaa gaa aaa tta ttt cca cat gta aca cca aaa | 1145 |
| | Asn Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His Val Thr Pro Lys | |
| | 220 225 230 | |
| 55 | gga att aat ggc ata gac ttt aaa ggg gaa gca ata act ttt aaa gca | 1193 |
| | Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala | |
| | 235 240 245 250 | |
| 60 | act act gct gga atc ctt gct aca ctt tct cat tgt att gaa tta atg | 1241 |
| | Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys Ile Glu Leu Met | |
| | 255 260 265 | |
| 65 | gta aaa cgg gaa gag agc tgg caa aaa aga cac gat agg gaa gtg gaa | 1289 |
| | Val Lys Arg Glu Glu Ser Trp Gln Lys Arg His Asp Arg Glu Val Glu | |
| | 270 275 280 | |
| 70 | aag agg aga cga gtg gag gaa gcg tac aag aat gtg atg gaa gaa ctt | 1337 |
| | Lys Arg Arg Arg Val Glu Glu Ala Tyr Lys Asn Val Met Glu Glu Leu | |
| | 285 290 295 | |
| 75 | aag aag aaa ccc cgt ttc gga ggg ccg gat tat gaa gaa ggt cca aac | 1385 |

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| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| | Lys | Lys | Lys | Pro | Arg | Phe | Gly | Gly | Pro | Asp | Tyr | Glu | Glu | Gly | Pro | Asn | |
| | 300 | | | | | | 305 | | | | | 310 | | | | | |
| 5 | agt | ctg | att | aat | gag | gaa | gag | ttc | ttt | gat | gct | ggt | gaa | gct | gct | ctt | 1433 |
| | Ser | Leu | Ile | Asn | Glu | Glu | Glu | Phe | Phe | Asp | Ala | Val | Glu | Ala | Ala | Leu | |
| | 315 | | | | 320 | | | | | 325 | | | | | 330 | | |
| 10 | gac | aga | caa | gat | aaa | ata | gag | gaa | cag | tca | cag | agt | gaa | aag | gtc | agg | 1481 |
| | Asp | Arg | Gln | Asp | Lys | Ile | Glu | Glu | Gln | Ser | Gln | Ser | Glu | Lys | Val | Arg | |
| | | | | | 335 | | | | | 340 | | | | | 345 | | |
| 15 | tta | cac | tgg | ccc | aca | tca | ttg | cca | tct | gga | gac | acc | ttt | tct | tct | gtc | 1529 |
| | Leu | His | Trp | Pro | Thr | Ser | Leu | Pro | Ser | Gly | Asp | Thr | Phe | Ser | Ser | Val | |
| | | | | 350 | | | | | 355 | | | | | 360 | | | |
| 20 | ggg | acg | cat | aga | ttt | gta | caa | aag | ccc | tat | agt | cgc | tct | tcc | tcc | atg | 1577 |
| | Gly | Thr | His | Arg | Phe | Val | Gln | Lys | Pro | Tyr | Ser | Arg | Ser | Ser | Ser | Met | |
| | | | 365 | | | | | 370 | | | | | 375 | | | | |
| 25 | tct | tcc | att | gat | cta | gtc | agt | gcc | tct | gac | gat | ggt | cac | aga | ttc | agc | 1625 |
| | Ser | Ser | Ile | Asp | Leu | Val | Ser | Ala | Ser | Asp | Asp | Val | His | Arg | Phe | Ser | |
| | | | 380 | | | | 385 | | | | | 390 | | | | | |
| 30 | tcc | cag | ggt | gaa | gaa | atg | gta | cag | aac | cac | atg | aac | tat | tca | tta | cag | 1673 |
| | Ser | Gln | Val | Glu | Glu | Met | Val | Gln | Asn | His | Met | Asn | Tyr | Ser | Leu | Gln | |
| | | | 395 | | | 400 | | | | | 405 | | | | | 410 | |
| 35 | gat | gta | ggt | ggt | gat | gca | aat | tgg | caa | ctg | ggt | ggt | gaa | gaa | gga | gaa | 1721 |
| | Asp | Val | Gly | Gly | Asp | Ala | Asn | Trp | Gln | Leu | Val | Val | Glu | Glu | Gly | Glu | |
| | | | | | 415 | | | | | 420 | | | | | 425 | | |
| 40 | atg | aag | gta | tac | aga | aga | gaa | gtg | gaa | gaa | aat | gga | att | ggt | ctg | gat | 1769 |
| | Met | Lys | Val | Tyr | Arg | Arg | Glu | Val | Glu | Glu | Asn | Gly | Ile | Val | Leu | Asp | |
| | | | | 430 | | | | | 435 | | | | 440 | | | | |
| 45 | cct | ttg | aaa | gct | act | cat | gca | ggt | aaa | ggt | ggt | aca | gga | cat | gag | gtc | 1817 |
| | Pro | Leu | Lys | Ala | Thr | His | Ala | Val | Lys | Gly | Val | Thr | Gly | His | Glu | Val | |
| | | | 445 | | | | | 450 | | | | | 455 | | | | |
| 50 | tgc | aat | tac | ttt | tgg | aat | ggt | gat | ggt | cgc | aat | gac | tgg | gaa | act | act | 1865 |
| | Cys | Asn | Tyr | Phe | Trp | Asn | Val | Asp | Val | Arg | Asn | Asp | Trp | Glu | Thr | Thr | |
| | | 460 | | | | 465 | | | | | | 470 | | | | | |
| 55 | ata | gaa | aac | ttt | cat | gtg | gtg | gaa | aca | tta | gct | gat | aat | gca | atc | atc | 1913 |
| | Ile | Glu | Asn | Phe | His | Val | Val | Glu | Thr | Leu | Ala | Asp | Asn | Ala | Ile | Ile | |
| | | 475 | | | | 480 | | | | | 485 | | | | 490 | | |
| 60 | ggt | tat | caa | acg | cac | aag | aga | gta | tgg | ccc | gct | tct | cag | aga | gac | gta | 1961 |
| | Val | Tyr | Gln | Thr | His | Lys | Arg | Val | Trp | Pro | Ala | Ser | Gln | Arg | Asp | Val | |
| | | | | | 495 | | | | | 500 | | | | | 505 | | |
| 65 | ctg | tat | ctt | tct | gct | att | cga | aag | atc | cca | gcc | ttg | act | gaa | aat | gat | 2009 |
| | Leu | Tyr | Leu | Ser | Ala | Ile | Arg | Lys | Ile | Pro | Ala | Leu | Thr | Glu | Asn | Asp | |
| | | | | 510 | | | | | 515 | | | | | 520 | | | |
| 70 | cct | gaa | act | tgg | ata | ggt | tgt | aat | ttt | tct | gtg | gat | cat | gat | agt | gct | 2057 |
| | Pro | Glu | Thr | Trp | Ile | Val | Cys | Asn | Phe | Ser | Val | Asp | His | Asp | Ser | Ala | |
| | | | 525 | | | | | 530 | | | | | 535 | | | | |
| 75 | cct | ctg | aac | aat | cga | tgt | gtc | cgt | gcc | aaa | atc | aat | att | gct | atg | att | 2105 |
| | Pro | Leu | Asn | Asn | Arg | Cys | Val | Arg | Ala | Lys | Ile | Asn | Ile | Ala | Met | Ile | |

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| | 540 | | 545 | | 550 | | |
|----|--|-----|-----|-----|-----|-----|------|
| 5 | tgt caa act tta gta agc cca cca gag gga gac cag gag ata agc aga | | | | | | 2153 |
| | Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asp Gln Glu Ile Ser Arg | | | | | | |
| | 555 | | 560 | | 565 | | 570 |
| 10 | gac aac att ctg tgc aag atc acg tat gta gct aat gtg aac cca gga | | | | | | 2201 |
| | Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly | | | | | | |
| | | 575 | | 580 | | 585 | |
| 15 | gga tgg gcg cca gct tcg gtc tta aga gca gtg gca aag cga gaa tac | | | | | | 2249 |
| | Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr | | | | | | |
| | | 590 | | 595 | | 600 | |
| 20 | cct aag ttt cta aaa cgt ttt act tct tat gtc caa gaa aaa act gca | | | | | | 2297 |
| | Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala | | | | | | |
| | | 605 | | 610 | | 615 | |
| 25 | gga aaa cca att ttg ttt tagtattaac agtgactgaa gcaaggctgc | | | | | | 2345 |
| | Gly Lys Pro Ile Leu Phe | | | | | | |
| | | 620 | | | | | |
| 30 | gtgacgttcc atgttggaga aaggagggaa aaaataaaaa gaatcctcta agctggaacg | | | | | | 2405 |
| | taggatctac agccttgtct gtggcccaag aagaaacatt gcaatcgtaa agctgggtat | | | | | | 2465 |
| | ccagcactag ccatctcctg ctaggcctcc tcgctcagcg tgtaactata aatacatgta | | | | | | 2525 |
| | gaatcacatg gatatggcta tattttttatt tgcttgctcc ttggagtgaa aacaaataac | | | | | | 2585 |
| 35 | tttgaattac aactaggaat taaccgatgc tttaattttg aggaactttt tcagaatttt | | | | | | 2645 |
| | ttatttacca tggccaacc taagatcctc agttgtatca agttttttgtg cacaaaagaa | | | | | | 2705 |
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 20 25 30
 Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
 35 40 45
 10 Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
 50 55 60
 Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65 70 75 80
 15 Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
 85 90 95

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Leu Arg Ala Gln Asp Pro Glu His Arg Gln Gln Trp Val Asp Ala Ile
 100 105 110
 5 Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
 115 120 125
 Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
 130 135 140
 10 Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
 145 150 155 160
 Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
 165 170 175
 15 Thr Leu Gln Lys Tyr Phe Asp Val Cys Ala Asp Ala Val Ser Lys Asp
 180 185 190
 Glu Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
 195 200 205
 20 Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
 210 215 220
 Glu Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
 225 230 235 240
 25 Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
 245 250 255
 Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Glu Ser
 260 265 270
 30 Trp Gln Lys Arg His Asp Arg Glu Val Glu Lys Arg Arg Arg Val Glu
 275 280 285
 Glu Ala Tyr Lys Asn Val Met Glu Glu Leu Lys Lys Lys Pro Arg Phe
 290 295 300
 35 Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
 305 310 315 320
 Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
 325 330 335
 40 Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser
 340 345 350
 Leu Pro Ser Gly Asp Thr Phe Ser Ser Val Gly Thr His Arg Phe Val
 355 360 365
 Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val
 370 375 380
 50 Ser Ala Ser Asp Asp Val His Arg Phe Ser Ser Gln Val Glu Glu Met
 385 390 395 400
 Val Gln Asn His Met Asn Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala
 405 410 415
 55 Asn Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg

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|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| | | | | 420 | | | | | 425 | | | | | 430 | | | |
| 5 | Glu | Val | Glu | Glu | Asn | Gly | Ile | Val | Leu | Asp | Pro | Leu | Lys | Ala | Thr | His | |
| | | | | 435 | | | | 440 | | | | | 445 | | | | |
| | Ala | Val | Lys | Gly | Val | Thr | Gly | His | Glu | Val | Cys | Asn | Tyr | Phe | Trp | Asn | |
| | | | | 450 | | | 455 | | | | | 460 | | | | | |
| 10 | Val | Asp | Val | Arg | Asn | Asp | Trp | Glu | Thr | Thr | Ile | Glu | Asn | Phe | His | Val | |
| | | | | | | 470 | | | | | 475 | | | | | 480 | |
| | Val | Glu | Thr | Leu | Ala | Asp | Asn | Ala | Ile | Ile | Val | Tyr | Gln | Thr | His | Lys | |
| | | | | | 485 | | | | | 490 | | | | | 495 | | |
| 15 | Arg | Val | Trp | Pro | Ala | Ser | Gln | Arg | Asp | Val | Leu | Tyr | Leu | Ser | Ala | Ile | |
| | | | | 500 | | | | | 505 | | | | | 510 | | | |
| | Arg | Lys | Ile | Pro | Ala | Leu | Thr | Glu | Asn | Asp | Pro | Glu | Thr | Trp | Ile | Val | |
| | | | | 515 | | | | 520 | | | | | 525 | | | | |
| 20 | Cys | Asn | Phe | Ser | Val | Asp | His | Asp | Ser | Ala | Pro | Leu | Asn | Asn | Arg | Cys | |
| | | | | | | | 535 | | | | | 540 | | | | | |
| | Val | Arg | Ala | Lys | Ile | Asn | Ile | Ala | Met | Ile | Cys | Gln | Thr | Leu | Val | Ser | |
| | | | | | | 550 | | | | | 555 | | | | | 560 | |
| 25 | Pro | Pro | Glu | Gly | Asp | Gln | Glu | Ile | Ser | Arg | Asp | Asn | Ile | Leu | Cys | Lys | |
| | | | | | 565 | | | | | 570 | | | | | 575 | | |
| | Ile | Thr | Tyr | Val | Ala | Asn | Val | Asn | Pro | Gly | Gly | Trp | Ala | Pro | Ala | Ser | |
| | | | | 580 | | | | | 585 | | | | | 590 | | | |
| | Val | Leu | Arg | Ala | Val | Ala | Lys | Arg | Glu | Tyr | Pro | Lys | Phe | Leu | Lys | Arg | |
| | | | | 595 | | | | 600 | | | | | 605 | | | | |
| 35 | Phe | Thr | Ser | Tyr | Val | Gln | Glu | Lys | Thr | Ala | Gly | Lys | Pro | Ile | Leu | Phe | |
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Met Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro
1 5 10 15

gag acg gag tcc ggg ccg ccg gtg gag cgc tgc gga gtc ctc aac aag 516
Glu Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Asn Lys
20 25 30

10 tgg aca aac tat att cat ggg tgg cag gat cgc tgg gta gtt ttg aaa 564
Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
35 40 45

15 aat aac act ctg agt tac tac aaa tct gaa gat gag aca gag tat ggc 612
Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
50 55 60

20 tgc aga gga tcc atc tgt ctt agc aag gct gtc atc acg cct cat gat 660
Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
65 70 75 80

25 ttt gat gaa tgc cga ttt gat att agt gta aat gat agt gtt tgg tat 708
Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
85 90 95

30 ctt cgt gct caa gat cca gat cac aga cag cag tgg ata gat gcc att 756
Leu Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile
100 105 110

35 gaa cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt 804
Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
115 120 125

40 cga cat ggc tcc atg gta tca ttg gta tcc gga gca agt ggc tat tct 852
Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
130 135 140

45 gca aca tcc acc tcc tca ttc aag aag ggc cac agt tta cgt gag aaa 900
Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
145 150 155 160

50 ctg gct gaa atg gaa acc ttt aga gat ata ctg tgt aga caa gtt gat 948
Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
165 170 175

55 acc cta cag aag ttc ttt gat gcc tgt gct gat gct gtc tcc aag gat 996
Thr Leu Gln Lys Phe Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp
180 185 190

gaa ttt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct 1044
Glu Phe Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
195 200 205

acg aca cgt tct gat gga gac ttc ttg cat aat acc aat ggc aat aag 1092
Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
210 215 220

gaa aag gta ttt cca cat gta aca cca aaa gga att aat ggt ata gac 1140
Glu Lys Val Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
225 230 235 240

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| | | |
|----|---|------|
| | ttt aaa ggt gag gcg ata act ttt aaa gca act act gcc gga atc ctt | 1188 |
| | Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu | |
| 5 | 245 250 255 | |
| | gct aca ctt tct cat tgt att gag ctg atg gta aaa cgt gag gac agc | 1236 |
| | Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser | |
| | 260 265 270 | |
| 10 | tgg caa aag aga atg gac aag gaa act gag aag aga aga aga gtg gag | 1284 |
| | Trp Gln Lys Arg Met Asp Lys Glu Thr Glu Lys Arg Arg Arg Val Glu | |
| | 275 280 285 | |
| 15 | gaa gca tac aaa aat gcc atg aca gaa ctt aag aaa aaa tcc cac ttt | 1332 |
| | Glu Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe | |
| | 290 295 300 | |
| 20 | gga gga cca gat tat gag gaa ggc cca aac agt ttg att aat gaa gag | 1380 |
| | Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu | |
| | 305 310 315 320 | |
| 25 | gag ttc ttt gat gct gtt gaa gct gct ctt gac aga caa gat aaa ata | 1428 |
| | Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile | |
| | 325 330 335 | |
| 30 | gaa gaa cag tcg cag agt gaa aag gtc agg tta cat tgg tct act tca | 1476 |
| | Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Ser Thr Ser | |
| | 340 345 350 | |
| 35 | atg cca tct gga gat gcc ttt tct tct gtg ggg act cat aga ttt gtc | 1524 |
| | Met Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val | |
| | 355 360 365 | |
| 40 | caa aag ccc tat agt cgc tct tcc tcc atg tct tcc att gat cta gtc | 1572 |
| | Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val | |
| | 370 375 380 | |
| 45 | agt gcc tct gac ggt gtt cac aga ttc agc tcc cag gtt gaa gag atg | 1620 |
| | Ser Ala Ser Asp Gly Val His Arg Phe Ser Ser Gln Val Glu Glu Met | |
| | 385 390 395 400 | |
| 50 | gtg cag aac cac atg acc tat tca ttg cag gat gta ggt ggg gac gcc | 1668 |
| | Val Gln Asn His Met Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala | |
| | 405 410 415 | |
| 55 | aac tgg cag ttg gtt gta gaa gaa ggg gag atg aag gta tat aga aga | 1716 |
| | Asn Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg | |
| | 420 425 430 | |
| 60 | gaa gta gaa gaa aat ggg att gtt ctg gat cct ttg aaa gct acc cat | 1764 |
| | Glu Val Glu Glu Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His | |
| | 435 440 445 | |
| 65 | gca gtt aaa ggc gtt aca gga cac gag gtc tgc aat tac ttc tgg aat | 1812 |
| | Ala Val Lys Gly Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn | |
| | 450 455 460 | |
| 70 | gtt gat gtt cgc aat gat tgg gaa aca act ata gaa aac ttt cat gtg | 1860 |
| | Val Asp Val Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val | |
| | 465 470 475 480 | |

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| | | |
|----|---|------|
| | gtg gaa aca tta gct gat aat gca atc atc att tat caa acg cac aag | 1908 |
| | Val Glu Thr Leu Ala Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys | |
| | 485 490 495 | |
| 5 | | |
| | aga gtg tgg cca gcc tct cag cgg gat gtc tta tat ctg tct gcc att | 1956 |
| | Arg Val Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Ala Ile | |
| | 500 505 510 | |
| 10 | | |
| | cga aag ata cca gct ttg aat gaa aat gac ccg gag act tgg ata gtt | 2004 |
| | Arg Lys Ile Pro Ala Leu Asn Glu Asn Asp Pro Glu Thr Trp Ile Val | |
| | 515 520 525 | |
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| | tgt aat ttt tct gta gat cac agc agt gct cct cta aac aat cga tgt | 2052 |
| | Cys Asn Phe Ser Val Asp His Ser Ser Ala Pro Leu Asn Asn Arg Cys | |
| | 530 535 540 | |
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| | gtc cgt gcc aaa ata aac gtt gct atg att tgt cag acc ttg gtg agc | 2100 |
| | Val Arg Ala Lys Ile Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser | |
| | 545 550 555 560 | |
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| | Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys | |
| | 565 570 575 | |
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| | att aca tac gtg gcc aat gta aac cct gga gga tgg gcc cca gcc tca | 2196 |
| | Ile Thr Tyr Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser | |
| | 580 585 590 | |
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| | gtg tta cgg gca gtg gca aag cga gaa tat cca aag ttt cta aag cgt | 2244 |
| | Val Leu Arg Ala Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg | |
| | 595 600 605 | |
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| | ttt act tct tac gta caa gaa aaa act gca gga aaa cct att ttg ttc | 2292 |
| | Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe | |
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Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
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Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
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|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
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| | Glu | Gln | His | Lys | Thr | Glu | Ser | Gly | Tyr | Gly | Ser | Glu | Ser | Ser | Leu | Arg |
| | | | 115 | | | | | 120 | | | | | 125 | | | |
| 10 | Arg | His | Gly | Ser | Met | Val | Ser | Leu | Val | Ser | Gly | Ala | Ser | Gly | Tyr | Ser |
| | | 130 | | | | | 135 | | | | | 140 | | | | |
| | Ala | Thr | Ser | Thr | Ser | Ser | Phe | Lys | Lys | Gly | His | Ser | Leu | Arg | Glu | Lys |
| 15 | 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| | Leu | Ala | Glu | Met | Glu | Thr | Phe | Arg | Asp | Ile | Leu | Cys | Arg | Gln | Val | Asp |
| | | | | 165 | | | | | | 170 | | | | | 175 | |
| | Thr | Leu | Gln | Lys | Phe | Phe | Asp | Ala | Cys | Ala | Asp | Ala | Val | Ser | Lys | Asp |
| 20 | | | | 180 | | | | | 185 | | | | | 190 | | |
| | Glu | Phe | Gln | Arg | Asp | Lys | Val | Val | Glu | Asp | Asp | Glu | Asp | Asp | Phe | Pro |
| | | | 195 | | | | | 200 | | | | | 205 | | | |
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| | Glu | Lys | Val | Phe | Pro | His | Val | Thr | Pro | Lys | Gly | Ile | Asn | Gly | Ile | Asp |
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| | Phe | Lys | Gly | Glu | Ala | Ile | Thr | Phe | Lys | Ala | Thr | Thr | Ala | Gly | Ile | Leu |
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| | | | | 260 | | | | | | 265 | | | | 270 | | |
| 35 | Trp | Gln | Lys | Arg | Met | Asp | Lys | Glu | Thr | Glu | Lys | Arg | Arg | Arg | Val | Glu |
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| 40 | Gly | Gly | Pro | Asp | Tyr | Glu | Glu | Gly | Pro | Asn | Ser | Leu | Ile | Asn | Glu | Glu |
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| | Glu | Phe | Phe | Asp | Ala | Val | Glu | Ala | Ala | Leu | Asp | Arg | Gln | Asp | Lys | Ile |
| 45 | | | | | 325 | | | | | 330 | | | | | 335 | |
| | Glu | Glu | Gln | Ser | Gln | Ser | Glu | Lys | Val | Arg | Leu | His | Trp | Ser | Thr | Ser |
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| | Gln | Lys | Pro | Tyr | Ser | Arg | Ser | Ser | Ser | Met | Ser | Ser | Ile | Asp | Leu | Val |
| | | 370 | | | | | 375 | | | | | 380 | | | | |
| 55 | Ser | Ala | Ser | Asp | Gly | Val | His | Arg | Phe | Ser | Ser | Gln | Val | Glu | Glu | Met |
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Val Gln Asn His Met Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala
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5 Asn Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg
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Val Asp Val Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val
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Val Glu Thr Leu Ala Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys
 485 490 495

Arg Val Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Ala Ile
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Arg Lys Ile Pro Ala Leu Asn Glu Asn Asp Pro Glu Thr Trp Ile Val
 515 520 525

25 Cys Asn Phe Ser Val Asp His Ser Ser Ala Pro Leu Asn Asn Arg Cys
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Val Arg Ala Lys Ile Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser
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Ile Thr Tyr Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser
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Met Ser Asp Asn Gln Ser Trp Asn
1 5
10 tcg tcg ggc tcg gag gag gat cca gag acg gag tct ggg ccg cct gtg 462
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15 gag cgc tgc ggg gtc ctc agt aag tgg aca aac tac att cat ggg tgg 510
Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn Tyr Ile His Gly Trp
25 30 35 40
20 cag gat cgt tgg gta gtt ttg aaa aat aat gct ctg agt tac tac aaa 558
Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala Leu Ser Tyr Tyr Lys
45 50 55
25 tct gaa gat gaa aca gag tat ggc tgc aga gga tcc atc tgt ctt agc 606
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30 aag gct gtc atc aca cct cac gat ttt gat gaa tgt cga ttt gat att 654
Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu Cys Arg Phe Asp Ile
75 80 85
35 agt gta aat gat agt gtt tgg tat ctt cgt gct cag gat cca gat cat 702
Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala Gln Asp Pro Asp His
90 95 100
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Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His Lys Thr Glu Ser Gly
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Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly Ser Met Val Ser Leu
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Val Ser Gly Ala Ser Gly Tyr Ser Ala Thr Ser Thr Ser Ser Phe Lys
140 145 150
55 aaa ggc cac agt tta cgt gag aag ttg gct gaa atg gaa aca ttt aga 894
Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg
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170 175 180
65 tgt gct gat gct gtc tct aag gat gaa ctt caa agg gat aaa gtg gta 990
Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val
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Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe
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| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| | Leu | His | Ser | Thr | Asn | Gly | Asn | Lys | Glu | Lys | Leu | Phe | Pro | His | Val | Thr | |
| | | | | 220 | | | | | 225 | | | | | 230 | | | |
| 5 | cca | aaa | gga | att | aat | ggt | ata | gac | ttt | aaa | ggg | gaa | gcg | ata | act | ttt | 1134 |
| | Pro | Lys | Gly | Ile | Asn | Gly | Ile | Asp | Phe | Lys | Gly | Glu | Ala | Ile | Thr | Phe | |
| | | | 235 | | | | | 240 | | | | | 245 | | | | |
| 10 | aaa | gca | act | act | gct | gga | atc | ctt | gca | aca | ctt | tct | cat | tgt | att | gaa | 1182 |
| | Lys | Ala | Thr | Thr | Ala | Gly | Ile | Leu | Ala | Thr | Leu | Ser | His | Cys | Ile | Glu | |
| | | 250 | | | | | 255 | | | | | 260 | | | | | |
| 15 | cta | atg | ggt | aaa | cgt | gag | gac | agc | tgg | cag | aag | aga | ctg | gat | aag | gaa | 1230 |
| | Leu | Met | Val | Lys | Arg | Glu | Asp | Ser | Trp | Gln | Lys | Arg | Leu | Asp | Lys | Glu | |
| | 265 | | | | | 270 | | | | | 275 | | | | | 280 | |
| 20 | act | gag | aag | aaa | aga | aga | aca | gag | gaa | gca | tat | aaa | aat | gca | atg | aca | 1278 |
| | Thr | Glu | Lys | Lys | Arg | Arg | Thr | Glu | Glu | Ala | Tyr | Lys | Asn | Ala | Met | Thr | |
| | | | | | 285 | | | | | 290 | | | | | 295 | | |
| 25 | gaa | ctt | aag | aaa | aaa | tcc | cac | ttt | gga | gga | cca | gat | tat | gaa | gaa | ggc | 1326 |
| | Glu | Leu | Lys | Lys | Lys | Ser | His | Phe | Gly | Gly | Pro | Asp | Tyr | Glu | Glu | Gly | |
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| 30 | cct | aac | agt | ctg | att | aat | gaa | gaa | gag | ttc | ttt | gat | gct | ggt | gaa | gct | 1374 |
| | Pro | Asn | Ser | Leu | Ile | Asn | Glu | Glu | Glu | Phe | Phe | Asp | Ala | Val | Glu | Ala | |
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| 35 | gct | ctt | gac | aga | caa | gat | aaa | ata | gaa | gaa | cag | tca | cag | agt | gaa | aag | 1422 |
| | Ala | Leu | Asp | Arg | Gln | Asp | Lys | Ile | Glu | Glu | Gln | Ser | Gln | Ser | Glu | Lys | |
| | | 330 | | | | | 335 | | | | | 340 | | | | | |
| 40 | gtg | aga | tta | cat | tgg | cct | aca | tcc | ttg | ccc | tct | gga | gat | gcc | ttt | tct | 1470 |
| | Val | Arg | Leu | His | Trp | Pro | Thr | Ser | Leu | Pro | Ser | Gly | Asp | Ala | Phe | Ser | |
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| 45 | tct | gtg | ggg | aca | cat | aga | ttt | gtc | caa | aag | gtt | gaa | gag | atg | gtg | cag | 1518 |
| | Ser | Val | Gly | Thr | His | Arg | Phe | Val | Gln | Lys | Val | Glu | Glu | Met | Val | Gln | |
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| 50 | aac | cac | atg | act | tac | tca | tta | cag | gat | gta | ggc | gga | gat | gcc | aat | tgg | 1566 |
| | Asn | His | Met | Thr | Tyr | Ser | Leu | Gln | Asp | Val | Gly | Gly | Asp | Ala | Asn | Trp | |
| | | | | 380 | | | | | 385 | | | | | 390 | | | |
| 55 | cag | ttg | ggt | gta | gaa | gaa | gga | gaa | atg | aag | gta | tac | aga | aga | gaa | gta | 1614 |
| | Gln | Leu | Val | Val | Glu | Glu | Gly | Glu | Met | Lys | Val | Tyr | Arg | Arg | Glu | Val | |
| | | | 395 | | | | | 400 | | | | | 405 | | | | |
| 60 | gaa | gaa | aat | ggg | att | gtt | ctg | gat | cct | tta | aaa | gct | acc | cat | gca | gtt | 1662 |
| | Glu | Glu | Asn | Gly | Ile | Val | Leu | Asp | Pro | Leu | Lys | Ala | Thr | His | Ala | Val | |
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| 65 | aaa | ggc | gtc | aca | gga | cat | gaa | gtc | tgc | aat | tat | ttc | tgg | aat | ggt | gac | 1710 |
| | Lys | Gly | Val | Thr | Gly | His | Glu | Val | Cys | Asn | Tyr | Phe | Trp | Asn | Val | Asp | |
| | 425 | | | | | 430 | | | | | 435 | | | | | 440 | |
| 70 | ggt | cgc | aat | gac | tgg | gaa | aca | act | ata | gaa | aac | ttt | cat | gtg | gtg | gaa | 1758 |
| | Val | Arg | Asn | Asp | Trp | Glu | Thr | Thr | Ile | Glu | Asn | Phe | His | Val | Val | Glu | |
| | | | | | 445 | | | | | 450 | | | | | 455 | | |
| 75 | aca | tta | gct | gat | aat | gca | atc | atc | att | tat | caa | aca | cac | aag | agg | gtg | 1806 |
| | Thr | Leu | Ala | Asp | Asn | Ala | Ile | Ile | Ile | Tyr | Gln | Thr | His | Lys | Arg | Val | |

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| | | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|------|
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| 5 | tgg | cct | gct | tct | cag | cga | gac | gta | tta | tat | ctt | tct | gtc | att | cga | aag | | 1854 |
| | Trp | Pro | Ala | Ser | Gln | Arg | Asp | Val | Leu | Tyr | Leu | Ser | Val | Ile | Arg | Lys | | |
| | | | 475 | | | | | 480 | | | | | 485 | | | | | |
| 10 | ata | cca | gcc | ttg | act | gaa | aat | gac | cct | gaa | act | tgg | ata | gtt | tgt | aat | | 1902 |
| | Ile | Pro | Ala | Leu | Thr | Glu | Asn | Asp | Pro | Glu | Thr | Trp | Ile | Val | Cys | Asn | | |
| | | | 490 | | | | 495 | | | | | 500 | | | | | | |
| 15 | ttt | tct | gtg | gat | cat | gac | agt | gct | cct | cta | aac | aac | cga | tgt | gtc | cgt | | 1950 |
| | Phe | Ser | Val | Asp | His | Asp | Ser | Ala | Pro | Leu | Asn | Asn | Arg | Cys | Val | Arg | | |
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| 20 | gcc | aaa | ata | aat | gtt | gct | atg | att | tgt | caa | acc | ttg | gta | agc | cca | cca | | 1998 |
| | Ala | Lys | Ile | Asn | Val | Ala | Met | Ile | Cys | Gln | Thr | Leu | Val | Ser | Pro | Pro | | |
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| 25 | gag | gga | aac | cag | gaa | att | agc | agg | gac | aac | att | cta | tgc | aag | att | aca | | 2046 |
| | Glu | Gly | Asn | Gln | Glu | Ile | Ser | Arg | Asp | Asn | Ile | Leu | Cys | Lys | Ile | Thr | | |
| | | | | 540 | | | | | 545 | | | | | 550 | | | | |
| 30 | tat | gta | gct | aat | gtg | aac | cct | gga | gga | tgg | gca | cca | gcc | tca | gtg | tta | | 2094 |
| | Tyr | Val | Ala | Asn | Val | Asn | Pro | Gly | Gly | Trp | Ala | Pro | Ala | Ser | Val | Leu | | |
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| 35 | agg | gca | gtg | gca | aag | cga | gag | tat | cct | aaa | ttt | cta | aaa | cgt | ttt | act | | 2142 |
| | Arg | Ala | Val | Ala | Lys | Arg | Glu | Tyr | Pro | Lys | Phe | Leu | Lys | Arg | Phe | Thr | | |
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| 40 | tct | tac | gtc | caa | gaa | aaa | act | gca | gga | aag | cct | att | ttg | ttc | tag | | | 2187 |
| | Ser | Tyr | Val | Gln | Glu | Lys | Thr | Ala | Gly | Lys | Pro | Ile | Leu | Phe | | | | |
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| 5 | Glu | Thr | Glu | Ser | Gly | Pro | Pro | Val | Glu | Arg | Cys | Gly | Val | Leu | Ser | Lys |
| | | | | 20 | | | | | 25 | | | | | 30 | | |
| | Trp | Thr | Asn | Tyr | Ile | His | Gly | Trp | Gln | Asp | Arg | Trp | Val | Val | Leu | Lys |
| | | | 35 | | | | | 40 | | | | | 45 | | | |
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| | | 50 | | | | | 55 | | | | | 60 | | | | |
| | Cys | Arg | Gly | Ser | Ile | Cys | Leu | Ser | Lys | Ala | Val | Ile | Thr | Pro | His | Asp |
| | 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| 15 | Phe | Asp | Glu | Cys | Arg | Phe | Asp | Ile | Ser | Val | Asn | Asp | Ser | Val | Trp | Tyr |
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 20 Glu Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
 225 230 235 240
 Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
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 30 Trp Gln Lys Arg Leu Asp Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu
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 Glu Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe
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 35 Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
 305 310 315 320
 Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
 325 330 335
 40 Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser
 340 345 350
 Leu Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val
 355 360 365
 45 Gln Lys Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser Leu Gln
 370 375 380
 Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu
 385 390 395 400
 50 Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp
 405 410 415
 55 Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
 420 425 430

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Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
 435 440 445
 5 Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
 450 455 460
 Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
 465 470 475 480
 10 Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp
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 Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp Ser Ala
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 15 Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala Met Ile
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 Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg
 530 535 540
 20 Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
 545 550 555 560
 Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
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gcgcaggggt cacggccacg gcggctgacg gctggaaggg caggctttct tcgccgctcg 240
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Met Ser Asp Asn Gln Ser Trp Asn Ser Ser
1 5 10

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ggc tcg gag gag gat ccg gag acg gag tcc ggg ccg cct gtg gag cgc 521
 Gly Ser Glu Glu Asp Pro Glu Thr Glu Ser Gly Pro Pro Val Glu Arg
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5 tgc ggg gtc ctc agc aag tgg aca aac tat att cat gga tgg cag gat 569
 Cys Gly Val Leu Ser Lys Trp Thr Asn Tyr Ile His Gly Trp Gln Asp
 30 35 40

10 cgt tgg gta gtt ttg aaa aat aat act ttg agt tac tac aaa tct gaa 617
 Arg Trp Val Val Leu Lys Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu
 45 50 55

15 gat gaa aca gaa tat ggc tgt agg gga tcc atc tgt ctt agc aag gct 665
 Asp Glu Thr Glu Tyr Gly Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala
 60 65 70

20 gtg atc acg cct cac gat ttt gat gaa tgc cgg ttt gat atc agt gta 713
 Val Ile Thr Pro His Asp Phe Asp Glu Cys Arg Phe Asp Ile Ser Val
 75 80 85 90

25 aat gat agt gtt tgg tac ctt cga gct cag gac ccg gag cac aga cag 761
 Asn Asp Ser Val Trp Tyr Leu Arg Ala Gln Asp Pro Glu His Arg Gln
 95 100 105

30 caa tgg gta gac gcc att gaa cag cac aag act gaa tcg gga tat gga 809
 Gln Trp Val Asp Ala Ile Glu Gln His Lys Thr Glu Ser Gly Tyr Gly
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35 tct gag tcc agc ttg cgt aga cat ggc tca atg gtg tca ctg gtg tct 857
 Ser Glu Ser Ser Leu Arg Arg His Gly Ser Met Val Ser Leu Val Ser
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40 gga gcg agt ggc tat tct gct acg tcc acc tct tct ttc aag aaa ggc 905
 Gly Ala Ser Gly Tyr Ser Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly
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45 cac agt tta cgt gag aaa ctg gct gaa atg gag aca ttt cgg gac atc 953
 His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg Asp Ile
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55 gac gct gtc tcc aag gat gag ctt cag agg gat aaa gtc gta gaa gat 1049
 Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val Glu Asp
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gat gaa gat gac ttc cct aca act cgt tct gat gga gac ttt ttg cac 1097
 Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe Leu His
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aat acc aat ggt aat aaa gaa aaa tta ttt cca cat gta aca cca aaa 1145
 Asn Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His Val Thr Pro Lys
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gga att aat ggc ata gac ttt aaa ggg gaa gca ata act ttt aaa gca 1193
 Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala
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| | | |
|----|---|------|
| | act act gct gga atc ctt gct aca ctt tct cat tgt att gaa tta atg | 1241 |
| | Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys Ile Glu Leu Met | |
| | 255 260 265 | |
| 5 | gta aaa cgg gaa gag agc tgg caa aaa aga cac gat agg gaa gtg gaa | 1289 |
| | Val Lys Arg Glu Glu Ser Trp Gln Lys Arg His Asp Arg Glu Val Glu | |
| | 270 275 280 | |
| 10 | aag agg aga cga gtg gag gaa gcg tac aag aat gtg atg gaa gaa ctt | 1337 |
| | Lys Arg Arg Arg Val Glu Glu Ala Tyr Lys Asn Val Met Glu Glu Leu | |
| | 285 290 295 | |
| 15 | aag aag aaa ccc cgt ttc gga ggg ccg gat tat gaa gaa ggt cca aac | 1385 |
| | Lys Lys Lys Pro Arg Phe Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn | |
| | 300 305 310 | |
| 20 | agt ctg att aat gag gaa gag ttc ttt gat gct gtt gaa gct gct ctt | 1433 |
| | Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu Ala Ala Leu | |
| | 315 320 325 330 | |
| 25 | gac aga caa gat aaa ata gag gaa cag tca cag agt gaa aag gtc agg | 1481 |
| | Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu Lys Val Arg | |
| | 335 340 345 | |
| 30 | tta cac tgg ccc aca tca ttg cca tct gga gac acc ttt tct tct gtc | 1529 |
| | Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Thr Phe Ser Ser Val | |
| | 350 355 360 | |
| 35 | ggg acg cat aga ttt gta caa aag gtt gaa gaa atg gta cag aac cac | 1577 |
| | Gly Thr His Arg Phe Val Gln Lys Val Glu Glu Met Val Gln Asn His | |
| | 365 370 375 | |
| 40 | atg aac tat tca tta cag gat gta ggt ggt gat gca aat tgg caa ctg | 1625 |
| | Met Asn Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln Leu | |
| | 380 385 390 | |
| 45 | gtt gtt gaa gaa gga gaa atg aag gta tac aga aga gaa gtg gaa gaa | 1673 |
| | Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu Glu | |
| | 395 400 405 410 | |
| 50 | aat gga att gtt ctg gat cct ttg aaa gct act cat gca gtt aaa ggt | 1721 |
| | Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys Gly | |
| | 415 420 425 | |
| 55 | gtt aca gga cat gag gtc tgc aat tac ttt tgg aat gtt gat gtt cgc | 1769 |
| | Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val Arg | |
| | 430 435 440 | |
| 60 | aat gac tgg gaa act act ata gaa aac ttt cat gtg gtg gaa aca tta | 1817 |
| | Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val Val Glu Thr Leu | |
| | 445 450 455 | |
| 65 | gct gat aat gca atc atc gtt tat caa acg cac aag aga gta tgg ccc | 1865 |
| | Ala Asp Asn Ala Ile Ile Val Tyr Gln Thr His Lys Arg Val Trp Pro | |
| | 460 465 470 | |
| 70 | gct tct cag aga gac gta ctg tat ctt tct gct att cga aag atc cca | 1913 |
| | Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Ala Ile Arg Lys Ile Pro | |
| | 475 480 485 490 | |
| 75 | gcc ttg act gaa aat gat cct gaa act tgg ata gtt tgt aat ttt tct | 1961 |

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| | | | | | | | | | | | | | | | | | | | | | | |
|----|-------|-------|-------|--------|------|------|--------|-------|------|-----|------|-------|------------|------|------|------|------|-------|------|------|---|------|
| | Ala | Leu | Thr | Glu | Asn | Asp | Pro | Glu | Thr | Trp | Ile | Val | Cys | Asn | Phe | Ser | | | | | | |
| | | | | | 495 | | | | | 500 | | | | | 505 | | | | | | | |
| 5 | gtg | gat | cat | gat | agt | gct | cct | ctg | aac | aat | cga | tgt | gtc | cgt | gcc | aaa | 2009 | | | | | |
| | Val | Asp | His | Asp | Ser | Ala | Pro | Leu | Asn | Asn | Arg | Cys | Val | Arg | Ala | Lys | | | | | | |
| | | | | 510 | | | | | 515 | | | | | 520 | | | | | | | | |
| 10 | atc | aat | att | gct | atg | att | tgt | caa | act | tta | gta | agc | cca | cca | gag | gga | 2057 | | | | | |
| | Ile | Asn | Ile | Ala | Met | Ile | Cys | Gln | Thr | Leu | Val | Ser | Pro | Pro | Glu | Gly | | | | | | |
| | | | | 525 | | | | 530 | | | | | | 535 | | | | | | | | |
| 15 | gac | cag | gag | ata | agc | aga | gac | aac | att | ctg | tgc | aag | atc | acg | tat | gta | 2105 | | | | | |
| | Asp | Gln | Glu | Ile | Ser | Arg | Asp | Asn | Ile | Leu | Cys | Lys | Ile | Thr | Tyr | Val | | | | | | |
| | | | 540 | | | | 545 | | | | | 550 | | | | | | | | | | |
| 20 | gct | aat | gtg | aac | cca | gga | gga | tgg | gcg | cca | gct | tcg | gtc | tta | aga | gca | 2153 | | | | | |
| | Ala | Asn | Val | Asn | Pro | Gly | Gly | Trp | Ala | Pro | Ala | Ser | Val | Leu | Arg | Ala | | | | | | |
| | | | | | | 555 | | 560 | | | 565 | | | | | 570 | | | | | | |
| 25 | gtg | gca | aag | cga | gaa | tac | cct | aag | ttt | cta | aaa | cgt | ttt | act | tct | tat | 2201 | | | | | |
| | Val | Ala | Lys | Arg | Glu | Tyr | Pro | Lys | Phe | Leu | Lys | Arg | Phe | Thr | Ser | Tyr | | | | | | |
| | | | | | 575 | | | | | 580 | | | | | | 585 | | | | | | |
| 30 | gtc | caa | gaa | aaa | act | gca | gga | aaa | cca | att | ttg | ttt | tagtattaac | | | | 2247 | | | | | |
| | Val | Gln | Glu | Lys | Thr | Ala | Gly | Lys | Pro | Ile | Leu | Phe | | | | | | | | | | |
| | | | | 590 | | | | | 595 | | | | | | | | | | | | | |
| 35 | agt | gact | gaa | gca | agg | ctgc | gtg | acg | ttcc | atg | ttg | gaga | aagg | aggg | gaa | aaa | ata | aaaaa | 2307 | | | |
| 40 | gaat | cct | cta | agct | gga | acg | tagg | at | ctac | agc | ctt | gtct | gtg | gcca | aag | aaga | aac | att | 2367 | | | |
| 45 | gcaat | cgtaa | agct | gggt | at | ccag | cact | ag | ccat | ctc | ctg | ctagg | cct | cc | tcg | ctc | agc | g | 2427 | | | |
| 50 | tgta | act | tata | aata | cat | gta | gaat | ca | cat | g | gat | at | gg | ct | tatt | ttt | tatt | tgct | tgct | 2487 | | |
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| 60 | agga | act | ttt | tcaga | at | ttt | ttatt | tacca | tggt | cca | acc | taag | at | cct | c | agtt | g | at | ca | 2607 | | |
| 65 | agtt | ttt | gtg | cacaaa | agaa | aag | cacaaa | g | ttg | aac | gca | cct | ga | agg | gca | tg | gct | ct | ct | 2667 | | |
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Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
35 40 45

10 Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
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15

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Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65 70 75 80
 5 Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
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 10 Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
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 Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
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 165 170 175
 20 Thr Leu Gln Lys Tyr Phe Asp Val Cys Ala Asp Ala Val Ser Lys Asp
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 Glu Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
 195 200 205
 25 Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
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 Glu Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
 225 230 235 240
 Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
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 Trp Gln Lys Arg His Asp Arg Glu Val Glu Lys Arg Arg Arg Val Glu
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 40 Glu Ala Tyr Lys Asn Val Met Glu Glu Leu Lys Lys Lys Pro Arg Phe
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 Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
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 355 360 365
 55 Gln Lys Val Glu Glu Met Val Gln Asn His Met Asn Tyr Ser Leu Gln
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Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu
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5 Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp
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Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
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10 Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
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Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
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15 Val Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
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Leu Tyr Leu Ser Ala Ile Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp
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20 Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp Ser Ala
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Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Ile Ala Met Ile
 515 520 525

Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asp Gln Glu Ile Ser Arg
 530 535 540

30 Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
 545 550 555 560

Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
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Gly Lys Pro Ile Leu Phe
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 aattcgggcg gcggcgccgg gcgcagcgca ggggtcacia cgacggcgac ggctgacggt 180
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 Glu Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Asn Lys
 20 25 30
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 Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
 35 40 45
 aat aac act ctg agt tac tac aaa tct gaa gat gag aca gag tat ggc 612
 Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
 50 55 60
 tgc aga gga tcc atc tgt ctt agc aag gct gtc atc acg cct cat gat 660
 Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65 70 75 80
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 Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
 85 90 95
 ctt cgt gct caa gat cca gat cac aga cag cag tgg ata gat gcc att 756
 Leu Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile
 100 105 110
 gaa cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt 804
 Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
 115 120 125
 cga cat ggc tcc atg gta tca ttg gta tcc gga gca agt ggc tat tct 852
 Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
 130 135 140
 gca aca tcc acc tcc tca ttc aag aag ggc cac agt tta cgt gag aaa 900
 Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
 145 150 155 160
 ctg gct gaa atg gaa acc ttt aga gat ata ctg tgt aga caa gtt gat 948
 Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
 165 170 175
 acc cta cag aag ttc ttt gat gcc tgt gct gat gct gtc tcc aag gat 996
 Thr Leu Gln Lys Phe Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp
 180 185 190
 gaa ttt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct 1044
 Glu Phe Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
 195 200 205
 acg aca cgt tct gat gga gac ttc ttg cat aat acc aat ggc aat aag 1092
 Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
 210 215 220

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| | gaa aag gta ttt cca cat gta aca cca aaa gga att aat ggt ata gac | 1140 |
| | Glu Lys Val Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp | |
| | 225 230 235 240 | |
| 5 | ttt aaa ggt gag gcg ata act ttt aaa gca act act gcc gga atc ctt | 1188 |
| | Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu | |
| | 245 250 255 | |
| 10 | gct aca ctt tct cat tgt att gag ctg atg gta aaa cgt gag gac agc | 1236 |
| | Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser | |
| | 260 265 270 | |
| 15 | tgg caa aag aga atg gac aag gaa act gag aag aga aga aga gtg gag | 1284 |
| | Trp Gln Lys Arg Met Asp Lys Glu Thr Glu Lys Arg Arg Arg Val Glu | |
| | 275 280 285 | |
| 20 | gaa gca tac aaa aat gcc atg aca gaa ctt aag aaa aaa tcc cac ttt | 1332 |
| | Glu Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe | |
| | 290 295 300 | |
| 25 | gga gga cca gat tat gag gaa ggc cca aac agt ttg att aat gaa gag | 1380 |
| | Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu | |
| | 305 310 315 320 | |
| 30 | gag ttc ttt gat gct gtt gaa gct gct ctt gac aga caa gat aaa ata | 1428 |
| | Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile | |
| | 325 330 335 | |
| 35 | gaa gaa cag tcg cag agt gaa aag gtc agg tta cat tgg tct act tca | 1476 |
| | Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Ser Thr Ser | |
| | 340 345 350 | |
| 40 | atg cca tct gga gat gcc ttt tct tct gtg ggg act cat aga ttt gtc | 1524 |
| | Met Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val | |
| | 355 360 365 | |
| 45 | caa aag gtt gaa gag atg gtg cag aac cac atg acc tat tca ttg cag | 1572 |
| | Gln Lys Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser Leu Gln | |
| | 370 375 380 | |
| 50 | gat gta ggt ggg gac gcc aac tgg cag ttg gtt gta gaa gaa ggg gag | 1620 |
| | Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu | |
| | 385 390 395 400 | |
| 55 | atg aag gta tat aga aga gaa gta gaa gaa aat ggg att gtt ctg gat | 1668 |
| | Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp | |
| | 405 410 415 | |
| 60 | cct ttg aaa gct acc cat gca gtt aaa ggc gtt aca gga cac gag gtc | 1716 |
| | Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val | |
| | 420 425 430 | |
| 65 | tgc aat tac ttc tgg aat gtt gat gtt cgc aat gat tgg gaa aca act | 1764 |
| | Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr | |
| | 435 440 445 | |
| 70 | ata gaa aac ttt cat gtg gtg gaa aca tta gct gat aat gca atc atc | 1812 |
| | Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile | |
| | 450 455 460 | |

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att tat caa acg cac aag aga gtg tgg cca gcc tct cag cgg gat gtc 1860
 Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
 465 470 475 480
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 Leu Tyr Leu Ser Ala Ile Arg Lys Ile Pro Ala Leu Asn Glu Asn Asp
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 Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
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| 15 | Leu | Ala | Glu | Met | Glu | Thr | Phe | Arg | Asp | Ile | Leu | Cys | Arg | Gln | Val | Asp |
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| 25 | Glu | Lys | Val | Phe | Pro | His | Val | Thr | Pro | Lys | Gly | Ile | Asn | Gly | Ile | Asp |
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| | Phe | Lys | Gly | Glu | Ala | Ile | Thr | Phe | Lys | Ala | Thr | Thr | Ala | Gly | Ile | Leu |
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| 35 | Glu | Ala | Tyr | Lys | Asn | Ala | Met | Thr | Glu | Leu | Lys | Lys | Lys | Ser | His | Phe |
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| 40 | Glu | Phe | Phe | Asp | Ala | Val | Glu | Ala | Ala | Leu | Asp | Arg | Gln | Asp | Lys | Ile |
| | | | | 325 | | | | | | 330 | | | | | 335 | |
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Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
 420 425 430

5 Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
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Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
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10 Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
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Leu Tyr Leu Ser Ala Ile Arg Lys Ile Pro Ala Leu Asn Glu Asn Asp
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15 Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Ser Ser Ala
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Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala Met Ile
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20 Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg
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25 Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
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Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
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 Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu

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acg gag tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg 147
 Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp

35 40 45

aca aac tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat 195
 Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn

50 55 60

aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc 243
 Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys

65 70 75

aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt 291
 Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe

80 85 90

gat gaa tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt 339
 Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu

95 100 105 110

cgt gct cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa 387
 Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu

115 120 125

cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga 435
 Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg

130 135 140

cat ggc tca atg gtg tcc ctg gtg tct gga gca agt ggc tac tct gca 483
 His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala

145 150 155

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5 gct gaa atg gaa aca ttt aga gac atc tta tgt aga caa gtt gac acg 579
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10 cta cag aag tac ttt gat gcc tgt gct gat gct gtc tct aag gat gaa 627
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15 ctt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct aca 675
 Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr
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20 aag tta ttt cca cat gtg aca cca aaa gga att aat ggt ata gac ttt 771
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25 aaa ggg gaa gcg ata act ttt aaa gca act act gct gga atc ctt gca 819
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30 aca ctt tct cat tgt att gaa cta atg gtt aaa cgt gag gac agc tgg 867
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35 gca tat aaa aat gca atg aca gaa cga aaa aat ccc act ttg gag gac 963
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gaagaaggag aaatgaaggat atacagaaga gaagtagaag aaaatgggat tgttctggat 1370

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Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
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Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
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Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
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Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
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Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
 100 105 110

Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
 20 115 120 125

Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
 25 130 135 140

Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala Thr Ser
 145 150 155 160

Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu
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| 10 | Arg | Asp | Lys | Val | Val | Glu | Asp | Asp | Glu | Asp | Asp | Phe | Pro | Thr | Thr | Arg |
| | | 210 | | | | | 215 | | | | | 220 | | | | |
| | Ser | Asp | Gly | Asp | Phe | Leu | His | Ser | Thr | Asn | Gly | Asn | Lys | Glu | Lys | Leu |
| | 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| 15 | Phe | Pro | His | Val | Thr | Pro | Lys | Gly | Ile | Asn | Gly | Ile | Asp | Phe | Lys | Gly |
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| | Glu | Ala | Ile | Thr | Phe | Lys | Ala | Thr | Thr | Ala | Gly | Ile | Leu | Ala | Thr | Leu |
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| | | |
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| | aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc | 243 |
| | Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys | |
| | 65 70 75 | |
| 5 | aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt | 291 |
| | Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe | |
| | 80 85 90 | |
| 10 | gat gaa tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt | 339 |
| | Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu | |
| | 95 100 105 110 | |
| 15 | cgt gct cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa | 387 |
| | Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu | |
| | 115 120 125 | |
| 20 | cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga | 435 |
| | Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg | |
| | 130 135 140 | |
| 25 | aca tcc acc tct tca ttc aag aaa ggc cac agt tta cgt gag aag ttg | 531 |
| | Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu | |
| | 160 165 170 | |
| 30 | gct gaa atg gaa aca ttt aga gac atc tta tgt aga caa gtt gac acg | 579 |
| | Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp Thr | |
| | 175 180 185 190 | |
| 35 | cta cag aag tac ttt gat gcc tgt gct gat gct gtc tct aag gat gaa | 627 |
| | Leu Gln Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu | |
| | 195 200 205 | |
| 40 | ctt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct aca | 675 |
| | Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr | |
| | 210 215 220 | |
| 45 | acg cgt tct gat ggt gac ttc ttg cat agt acc aac ggc aat aaa gaa | 723 |
| | Thr Arg Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu | |
| | 225 230 235 | |
| 50 | aag tta ttt cca cat gtg aca cca aaa gga att aat ggt ata gac ttt | 771 |
| | Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe | |
| | 240 245 250 | |
| 55 | aaa ggg gaa gcg ata act ttt aaa gca act act gct gga atc ctt gca | 819 |
| | Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala | |
| | 255 260 265 270 | |
| 60 | aca ctt tct cat tgt att gaa cta atg gtt aaa cgt gag gac agc tgg | 867 |
| | Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp | |
| | 275 280 285 | |
| 65 | cag aag aga ctg gat aag gaa act gag cac ttt gga gga cca gat tat | 915 |
| | Gln Lys Arg Leu Asp Lys Glu Thr Glu His Phe Gly Gly Pro Asp Tyr | |
| | 290 295 300 | |

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| | | |
|----|---|------|
| | gaa gaa ggc cct aac agt ctg att aat gaa gaa gag ttc ttt gat gct | 963 |
| | Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala | |
| | 305 310 315 | |
| 5 | gtt gaa gct gct ctt gac aga caa gat aaa ata gaa gaa cag tca cag | 1011 |
| | Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln | |
| | 320 325 330 | |
| 10 | agt gaa aag gtg aga tta cat tgg cct aca tcc ttg ccc tct gga gat | 1059 |
| | Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp | |
| | 335 340 345 350 | |
| 15 | gcc ttt tct tct gtg ggg aca cat aga ttt gtc caa aag ccc tat agt | 1107 |
| | Ala Phe Ser Ser Val Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser | |
| | 355 360 365 | |
| 20 | cgc tct tcc tcc atg tct tcc att gat cta gtc agt gcc tct gat gat | 1155 |
| | Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp | |
| | 370 375 380 | |
| 25 | gta gaa gaa gga gaa atg aag gta tac aga aga gaa gta gaa gaa aat | 1299 |
| | Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn | |
| | 415 420 425 430 | |
| 30 | ggg att gtt ctg gat cct tta aaa gct acc cat gca gtt aaa ggc gtc | 1347 |
| | Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys Gly Val | |
| | 435 440 445 | |
| 35 | aca gga cat gaa gtc tgc aat tat ttc tgg aat gtt gac gtt cgc aat | 1395 |
| | Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn | |
| | 450 455 460 | |
| 40 | gac tgg gaa aca act ata gaa aac ttt cat gtg gtg gaa aca tta gct | 1443 |
| | Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val Val Glu Thr Leu Ala | |
| | 465 470 475 | |
| 45 | gat aat gca atc atc att tat caa aca cac aag agg gtg tgg cct gct | 1491 |
| | Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala | |
| | 480 485 490 | |
| 50 | tct cag cga gac gta tta tat ctt tct gtc att cga aag ata cca gcc | 1539 |
| | Ser Gln Arg Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala | |
| | 495 500 505 510 | |
| 55 | ttg act gaa aat gac cct gaa act tgg ata gtt tgt aat ttt tct gtg | 1587 |
| | Leu Thr Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val | |
| | 515 520 525 | |
| 55 | gat cat gac agt gct cct cta aac aac cga tgt gtc cgt gcc aaa ata | 1635 |
| | Asp His Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile | |
| | 530 535 540 | |
| 55 | aat gtt gct atg att tgt caa acc ttg gta agc cca cca gag gga aac | 1683 |

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| | | | | | | | | | | | | | | | | | |
|----|---------|--|--------|---------|---------|-------|--------|------|--------|------|--------|------------|------------|------|-----|-----|------|
| | Asn | Val | Ala | Met | Ile | Cys | Gln | Thr | Leu | Val | Ser | Pro | Pro | Glu | Gly | Asn | |
| | | | 545 | | | | | 550 | | | | | 555 | | | | |
| 5 | cag | gaa | att | agc | agg | gac | aac | att | cta | tgc | aag | att | aca | tat | gta | gct | 1731 |
| | Gln | Glu | Ile | Ser | Arg | Asp | Asn | Ile | Leu | Cys | Lys | Ile | Thr | Tyr | Val | Ala | |
| | | 560 | | | | | 565 | | | | 570 | | | | | | |
| 10 | aat | gtg | aac | cct | gga | gga | tgg | gca | cca | gcc | tca | gtg | tta | agg | gca | gtg | 1779 |
| | Asn | Val | Asn | Pro | Gly | Gly | Trp | Ala | Pro | Ala | Ser | Val | Leu | Arg | Ala | Val | |
| | | 575 | | | | 580 | | | | | 585 | | | | | 590 | |
| 15 | gca | aag | cga | gag | tat | cct | aaa | ttt | cta | aaa | cgt | ttt | act | tct | tac | gtc | 1827 |
| | Ala | Lys | Arg | Glu | Tyr | Pro | Lys | Phe | Leu | Lys | Arg | Phe | Thr | Ser | Tyr | Val | |
| | | | | 595 | | | | | 600 | | | | | | 605 | | |
| 20 | caa | gaa | aaa | act | gca | gga | aag | cct | att | ttg | ttc | tagtattaac | aggtactaga | 1880 | | | |
| | Gln | Glu | Lys | Thr | Ala | Gly | Lys | Pro | Ile | Leu | Phe | | | | | | |
| | | | | 610 | | | | | 615 | | | | | | | | |
| 25 | agatatg | ttt | tat | ctttttt | taacttt | tatt | tgacta | atat | gactgt | caat | actaaa | attt | 1940 | | | | |
| | agttgt | tgaa | agtatt | tact | atgttt | ttttc | cgga | attc | | | | | 1978 | | | | |
| 30 | <210> | 18 | | | | | | | | | | | | | | | |
| | <211> | 617 | | | | | | | | | | | | | | | |
| | <212> | PRT | | | | | | | | | | | | | | | |
| | <213> | Artificial Sequence | | | | | | | | | | | | | | | |
| 35 | <220> | | | | | | | | | | | | | | | | |
| | <223> | Description of Artificial Sequence: FLAG-GPBDNLS | | | | | | | | | | | | | | | |
| 40 | <400> | 18 | | | | | | | | | | | | | | | |

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

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Ala Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln
 485 490 495

5 Arg Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr
 500 505 510

Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His
 515 520 525

10 Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val
 530 535 540

Ala Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu
 545 550 555 560

15 Ile Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val
 565 570 575

Asn Pro Gly Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys
 580 585 590

20 Arg Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu
 595 600 605

Lys Thr Ala Gly Lys Pro Ile Leu Phe
 610 615

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<210> 19
 <211> 1975
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gaattcacc atg gcc cca cta gcc gac tac aag gac gac gat gac aag atg 51
 Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met
 1 5 10

tcg gat aat cag agc tgg aac tcg tcg ggc tcg gag gag gat cca gag 99
 Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu
 15 20 25 30

acg gag tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg 147
 Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp
 35 40 45

aca aac tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat 195
 Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn
 50 55 60

aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc 243
 Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys
 65 70 75

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| | | |
|----|---|-----|
| | aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt | 291 |
| | Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe | |
| | 80 85 90 | |
| 5 | gat gaa tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt | 339 |
| | Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu | |
| | 95 100 105 110 | |
| 10 | cgt gct cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa | 387 |
| | Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu | |
| | 115 120 125 | |
| 15 | cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga | 435 |
| | Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg | |
| | 130 135 140 | |
| 20 | cat ggc aaa ggc cac agt tta cgt gag aag ttg gct gaa atg gaa aca | 483 |
| | His Gly Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr | |
| | 145 150 155 | |
| 25 | ttt aga gac atc tta tgt aga caa gtt gac acg cta cag aag tac ttt | 531 |
| | Phe Arg Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe | |
| | 160 165 170 | |
| 30 | gat gcc tgt gct gat gct gtc tct aag gat gaa ctt caa agg gat aaa | 579 |
| | Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys | |
| | 175 180 185 190 | |
| 35 | gtg gta gaa gat gat gaa gat gac ttt cct aca acg cgt tct gat ggt | 627 |
| | Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly | |
| | 195 200 205 | |
| 40 | gac ttc ttg cat agt acc aac ggc aat aaa gaa aag tta ttt cca cat | 675 |
| | Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His | |
| | 210 215 220 | |
| 45 | gtg aca cca aaa gga att aat ggt ata gac ttt aaa ggg gaa gcg ata | 723 |
| | Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile | |
| | 225 230 235 | |
| 50 | act ttt aaa gca act act gct gga atc ctt gca aca ctt tct cat tgt | 771 |
| | Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys | |
| | 240 245 250 | |
| 55 | att gaa cta atg gtt aaa cgt gag gac agc tgg cag aag aga ctg gat | 819 |
| | Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys Arg Leu Asp | |
| | 255 260 265 270 | |
| 60 | aag gaa act gag aag aaa aga aga aca gag gaa gca tat aaa aat gca | 867 |
| | Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu Glu Ala Tyr Lys Asn Ala | |
| | 275 280 285 | |
| 65 | atg aca gaa ctt aag aaa aaa tcc cac ttt gga gga cca gat tat gaa | 915 |
| | Met Thr Glu Leu Lys Lys Lys Ser His Phe Gly Gly Pro Asp Tyr Glu | |
| | 290 295 300 | |
| 70 | gaa ggc cct aac agt ctg att aat gaa gaa gag ttc ttt gat gct gtt | 963 |
| | Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val | |
| | 305 310 315 | |

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| | | |
|----|---|------|
| | gaa gct gct ctt gac aga caa gat aaa ata gaa gaa cag tca cag agt | 1011 |
| | Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser | |
| | 320 325 330 | |
| 5 | gaa aag gtg aga tta cat tgg cct aca tcc ttg ccc tct gga gat gcc | 1059 |
| | Glu Lys Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Ala | |
| | 335 340 345 350 | |
| 10 | ttt tct tct gtg ggg aca cat aga ttt gtc caa aag ccc tat agt cgc | 1107 |
| | Phe Ser Ser Val Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser Arg | |
| | 355 360 365 | |
| 15 | tct tcc tcc atg tct tcc att gat cta gtc agt gcc tct gat gat gtt | 1155 |
| | Ser Ser Ser Met Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp Val | |
| | 370 375 380 | |
| 20 | cac aga ttc agc tcc cag gtt gaa gag atg gtg cag aac cac atg act | 1203 |
| | His Arg Phe Ser Ser Gln Val Glu Glu Met Val Gln Asn His Met Thr | |
| | 385 390 395 | |
| 25 | tac tca tta cag gat gta ggc gga gat gcc aat tgg cag ttg gtt gta | 1251 |
| | Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val | |
| | 400 405 410 | |
| 30 | gaa gaa gga gaa atg aag gta tac aga aga gaa gta gaa gaa aat ggg | 1299 |
| | Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly | |
| | 415 420 425 430 | |
| 35 | att gtt ctg gat cct tta aaa gct acc cat gca gtt aaa ggc gtc aca | 1347 |
| | Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr | |
| | 435 440 445 | |
| 40 | gga cat gaa gtc tgc aat tat ttc tgg aat gtt gac gtt cgc aat gac | 1395 |
| | Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp | |
| | 450 455 460 | |
| 45 | tgg gaa aca act ata gaa aac ttt cat gtg gtg gaa aca tta gct gat | 1443 |
| | Trp Glu Thr Thr Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp | |
| | 465 470 475 | |
| 50 | aat gca atc atc att tat caa aca cac aag agg gtg tgg cct gct tct | 1491 |
| | Asn Ala Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser | |
| | 480 485 490 | |
| 55 | cag cga gac gta tta tat ctt tct gtc att cga aag ata cca gcc ttg | 1539 |
| | Gln Arg Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu | |
| | 495 500 505 510 | |
| 60 | act gaa aat gac cct gaa act tgg ata gtt tgt aat ttt tct gtg gat | 1587 |
| | Thr Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp | |
| | 515 520 525 | |
| 65 | cat gac agt gct cct cta aac aac cga tgt gtc cgt gcc aaa ata aat | 1635 |
| | His Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn | |
| | 530 535 540 | |
| 70 | gtt gct atg att tgt caa acc ttg gta agc cca cca gag gga aac cag | 1683 |
| | Val Ala Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln | |
| | 545 550 555 | |
| 75 | gaa att agc agg gac aac att cta tgc aag att aca tat gta gct aat | 1731 |

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|----|---------|-------|--------|-------|-------|--------|-------|-------|--------|------|------------|------|---------|-----|-----|-----|------|
| | Glu | Ile | Ser | Arg | Asp | Asn | Ile | Leu | Cys | Lys | Ile | Thr | Tyr | Val | Ala | Asn | |
| | 560 | | | | | | 565 | | | | | 570 | | | | | |
| 5 | gtg | aac | cct | gga | gga | tgg | gca | cca | gcc | tca | gtg | tta | agg | gca | gtg | gca | 1779 |
| | Val | Asn | Pro | Gly | Gly | Trp | Ala | Pro | Ala | Ser | Val | Leu | Arg | Ala | Val | Ala | |
| | 575 | | | | | 580 | | | | | 585 | | | | | 590 | |
| 10 | aag | cga | gag | tat | cct | aaa | ttt | cta | aaa | cgt | ttt | act | tct | tac | gtc | caa | 1827 |
| | Lys | Arg | Glu | Tyr | Pro | Lys | Phe | Leu | Lys | Arg | Phe | Thr | Ser | Tyr | Val | Gln | |
| | | | | | 595 | | | | | 600 | | | | | 605 | | |
| 15 | gaa | aaa | act | gca | gga | aag | cct | att | ttg | ttc | tagtattaac | agg | tactaga | | | | 1877 |
| | Glu | Lys | Thr | Ala | Gly | Lys | Pro | Ile | Leu | Phe | | | | | | | |
| | | | | 610 | | | | | 615 | | | | | | | | |
| | agatatg | ttt | tatct | ttttt | taact | tttatt | tgact | aatat | gactgt | caat | actaaa | attt | | | | | 1937 |
| | agttg | ttgaa | agtatt | tact | atgt | tttttc | cgga | attc | | | | | | | | | 1975 |

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<210> 20
 <211> 616
 <212> PRT
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 <220>
 <223> Description of Artificial Sequence: FLAG-GPBPDSXY
 <400> 20

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Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met Ser Asp
 1 5 10 15

Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu
 5 20 25 30

Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
 35 40 45

Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
 10 50 55 60

Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
 65 70 75 80

Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
 15 85 90 95

Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
 100 105 110

Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
 20 115 120 125

Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
 25 130 135 140

Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg
 145 150 155 160

Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe Asp Ala
 30 165 170 175

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Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val
 180 185 190
 5 Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe
 195 200 205
 Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His Val Thr
 210 215 220
 10 Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile Thr Phe
 225 230 235 240
 Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys Ile Glu
 245 250 255
 15 Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys Arg Leu Asp Lys Glu
 260 265 270
 Thr Glu Lys Lys Arg Arg Thr Glu Glu Ala Tyr Lys Asn Ala Met Thr
 275 280 285
 20 Glu Leu Lys Lys Lys Ser His Phe Gly Gly Pro Asp Tyr Glu Glu Gly
 290 295 300
 Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu Ala
 305 310 315 320
 Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu Lys
 325 330 335
 30 Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Ala Phe Ser
 340 345 350
 Ser Val Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser Arg Ser Ser
 355 360 365
 35 Ser Met Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp Val His Arg
 370 375 380
 Phe Ser Ser Gln Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser
 385 390 395 400
 40 Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu
 405 410 415
 Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val
 420 425 430
 45 Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His
 435 440 445
 Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu
 450 455 460
 50 Thr Thr Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala
 465 470 475 480
 55 Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg
 485 490 495

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Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr Glu
 500 505 510
 5 Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp
 515 520 525
 Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala
 530 535 540
 10 Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile
 545 550 555 560
 Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn
 565 570 575
 15 Pro Gly Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg
 580 585 590
 Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys
 595 600 605
 20 Thr Ala Gly Lys Pro Ile Leu Phe
 610 615
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 <222> (10)..(1797)
 35 <400> 21

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 Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met
 1 5 10
 40 tcg gat aat cag agc tgg aac tcg tcg ggc tcg gag gag gat cca gag 99
 Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu
 15 20 25 30
 45 acg gag tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg 147
 Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp
 35 40 45
 50 aca aac tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat 195
 Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn
 50 55 60
 aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc 243
 Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys
 65 70 75
 55 aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt 291
 Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe

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| | 80 | | 85 | | 90 | | |
|----|---------------------|---------------------|---------------------|-----|------|--|--|
| 5 | gat gaa tgt cga ttt | gat att agt gta aat | gat agt gtt tgg tat | ctt | 339 | | |
| | Asp Glu Cys Arg Phe | Asp Ile Ser Val Asn | Asp Ser Val Trp Tyr | Leu | | | |
| | 95 | 100 | 105 | 110 | | | |
| 10 | cgt gct cag gat cca | gat cat aga cag caa | tgg ata gat gcc att | gaa | 387 | | |
| | Arg Ala Gln Asp Pro | Asp His Arg Gln Gln | Trp Ile Asp Ala Ile | Glu | | | |
| | | 115 | 120 | 125 | | | |
| 15 | cag cac aag act gaa | tct gga tat gga tct | gaa tcc agc ttg cgt | cga | 435 | | |
| | Gln His Lys Thr Glu | Ser Gly Tyr Gly Ser | Glu Ser Ser Leu Arg | Arg | | | |
| | | 130 | 135 | 140 | | | |
| 20 | cat ggc aaa ggc cac | agt tta cgt gag aag | ttg gct gaa atg gaa | aca | 483 | | |
| | His Gly Lys Gly His | Ser Leu Arg Glu Lys | Leu Ala Glu Met Glu | Thr | | | |
| | | 145 | 150 | 155 | | | |
| 25 | ttt aga gac atc tta | tgt aga caa gtt gac | acg cta cag aag tac | ttt | 531 | | |
| | Phe Arg Asp Ile Leu | Cys Arg Gln Val Asp | Thr Leu Gln Lys Tyr | Phe | | | |
| | | 160 | 165 | 170 | | | |
| 30 | gat gcc tgt gct gat | gct gtc tct aag gat | gaa ctt caa agg gat | aaa | 579 | | |
| | Asp Ala Cys Ala Asp | Ala Val Ser Lys Asp | Glu Leu Gln Arg Asp | Lys | | | |
| | | 175 | 180 | 185 | 190 | | |
| 35 | gtg gta gaa gat gat | gaa gat gac ttt cct | aca acg cgt tct gat | ggg | 627 | | |
| | Val Val Glu Asp Asp | Glu Asp Asp Phe Pro | Thr Thr Arg Ser Asp | Gly | | | |
| | | 195 | 200 | 205 | | | |
| 40 | gac ttc ttg cat agt | acc aac ggc aat aaa | gaa aag tta ttt cca | cat | 675 | | |
| | Asp Phe Leu His Ser | Thr Asn Gly Asn Lys | Glu Lys Leu Phe Pro | His | | | |
| | | 210 | 215 | 220 | | | |
| 45 | gtg aca cca aaa gga | att aat ggt ata gac | ttt aaa ggg gaa gcg | ata | 723 | | |
| | Val Thr Pro Lys Gly | Ile Asn Gly Ile Asp | Phe Lys Gly Glu Ala | Ile | | | |
| | | 225 | 230 | 235 | | | |
| 50 | act ttt aaa gca act | act gct gga atc ctt | gca aca ctt tct cat | tgt | 771 | | |
| | Thr Phe Lys Ala Thr | Thr Ala Gly Ile Leu | Ala Thr Leu Ser His | Cys | | | |
| | | 240 | 245 | 250 | | | |
| 55 | att gaa cta atg gtt | aaa cgt gag gac agc | tgg cag aag aga ctg | gat | 819 | | |
| | Ile Glu Leu Met Val | Lys Arg Glu Asp Ser | Trp Gln Lys Arg Leu | Asp | | | |
| | | 255 | 260 | 270 | | | |
| 60 | aag gaa act gag cac | ttt gga gga cca gat | tat gaa gaa ggc cct | aac | 867 | | |
| | Lys Glu Thr Glu His | Phe Gly Gly Pro Asp | Tyr Glu Glu Gly Pro | Asn | | | |
| | | 275 | 280 | 285 | | | |
| 65 | agt ctg att aat gaa | gaa gag ttc ttt gat | gct gtt gaa gct gct | ctt | 915 | | |
| | Ser Leu Ile Asn Glu | Glu Glu Phe Phe Asp | Ala Val Glu Ala Ala | Leu | | | |
| | | 290 | 295 | 300 | | | |
| 70 | gac aga caa gat aaa | ata gaa gaa cag tca | cag agt gaa aag gtg | aga | 963 | | |
| | Asp Arg Gln Asp Lys | Ile Glu Glu Gln Ser | Gln Ser Glu Lys Val | Arg | | | |
| | | 305 | 310 | 315 | | | |
| 75 | tta cat tgg cct aca | tcc ttg ccc tct gga | gat gcc ttt tct tct | gtg | 1011 | | |
| | Leu His Trp Pro Thr | Ser Leu Pro Ser Gly | Asp Ala Phe Ser Ser | Val | | | |
| | | 320 | 325 | 330 | | | |

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ggg aca cat aga ttt gtc caa aag ccc tat agt cgc tct tcc tcc atg 1059
 Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met
 335 340 345 350

tct tcc att gat cta gtc agt gcc tct gat gat gtt cac aga ttc agc 1107
 Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp Val His Arg Phe Ser
 355 360 365

tcc cag gtt gaa gag atg gtg cag aac cac atg act tac tca tta cag 1155
 Ser Gln Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser Leu Gln
 370 375 380

gat gta ggc gga gat gcc aat tgg cag ttg gtt gta gaa gaa gga gaa 1203
 Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu
 385 390 395

atg aag gta tac aga aga gaa gta gaa gaa aat ggg att gtt ctg gat 1251
 Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp
 400 405 410

cct tta aaa gct acc cat gca gtt aaa ggc gtc aca gga cat gaa gtc 1299
 Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
 415 420 425 430

tgc aat tat ttc tgg aat gtt gac gtt cgc aat gac tgg gaa aca act 1347
 Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
 435 440 445

ata gaa aac ttt cat gtg gtg gaa aca tta gct gat aat gca atc atc 1395
 Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
 450 455 460

att tat caa aca cac aag agg gtg tgg cct gct tct cag cga gac gta 1443
 Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
 465 470 475

tta tat ctt tct gtc att cga aag ata cca gcc ttg act gaa aat gac 1491
 Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp
 480 485 490

cct gaa act tgg ata gtt tgt aat ttt tct gtg gat cat gac agt gct 1539
 Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp Ser Ala
 495 500 505 510

cct cta aac aac cga tgt gtc cgt gcc aaa ata aat gtt gct atg att 1587
 Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala Met Ile
 515 520 525

tgt caa acc ttg gta agc cca cca gag gga aac cag gaa att agc agg 1635
 Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg
 530 535 540

gac aac att cta tgc aag att aca tat gta gct aat gtg aac cct gga 1683
 Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
 545 550 555

gga tgg gca cca gcc tca gtg tta agg gca gtg gca aag cga gag tat 1731
 Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
 560 565 570

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5 cct aaa ttt cta aaa cgt ttt act tct tac gtc caa gaa aaa act gca 1779
Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala
575 580 585 590

gga aag cct att ttg ttc tagtattaac aggtactaga agatatgttt 1827
Gly Lys Pro Ile Leu Phe
595

10 tatctttttt taactttatt tgactaatat gactgtcaat actaaaattt agttggtgaa 1887
agtatttact atgttttttc cggaattc 1915

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Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met Ser Asp
 1 5 10 15

5 Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu
 20 25 30

Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
 35 40 45

10 Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
 50 55 60

Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
 65 70 75 80

15 Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
 85 90 95

Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
 100 105 110

20 Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
 115 120 125

Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
 130 135 140

25 Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg
 145 150 155 160

30 Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe Asp Ala
 165 170 175

Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val
 180 185 190

35 Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe
 195 200 205

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5 Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His Val Thr
 210 215 220
 Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile Thr Phe
 225 230 235 240
 10 Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys Ile Glu
 245 250 255
 Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys Arg Leu Asp Lys Glu
 260 265 270
 15 Thr Glu His Phe Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu
 275 280 285
 Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg
 290 295 300
 20 Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His
 305 310 315 320
 Trp Pro Thr Ser Leu Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr
 325 330 335
 25 His Arg Phe Val Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser
 340 345 350
 Ile Asp Leu Val Ser Ala Ser Asp Asp Val His Arg Phe Ser Ser Gln
 355 360 365
 30 Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser Leu Gln Asp Val
 370 375 380
 Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys
 385 390 395 400
 35 Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp Pro Leu
 405 410 415
 Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val Cys Asn
 420 425 430
 40 Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr Ile Glu
 435 440 445
 Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile Ile Tyr
 450 455 460
 45 Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr
 465 470 475 480
 50 Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp Pro Glu
 485 490 495
 Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp Ser Ala Pro Leu
 500 505 510
 55 Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala Met Ile Cys Gln
 515 520 525

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Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg Asp Asn
 530 535 540

5 Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly Gly Trp
 545 550 555 560

Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr Pro Lys
 565 570 575

10 Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys
 580 585 590

Pro Ile Leu Phe
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 Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Lys Met
 1 5 10

tcg gat aat cag agc tgg aac tcg tcg ggc tcg gag gag gat cca gag 99
 Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu
 35 15 20 25 30

acg gag tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg 147
 Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp
 35 40 45

40 aca aac tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat 195
 Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn
 50 55 60

45 aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc 243
 Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys
 65 70 75

50 aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt 291
 Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe
 80 85 90

gat gaa tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt 339
 Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu
 95 100 105 110

55 cgt gct cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa 387
 Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu
 115 120 125

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| | | |
|----|---|------|
| | cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga | 435 |
| | Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg | |
| | 130 135 140 | |
| 5 | cat ggc tca atg gtg tcc ctg gtg tct gga gca agt ggc tac tct gca | 483 |
| | His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala | |
| | 145 150 155 | |
| 10 | aca tcc acc tct tca ttc aag aaa ggc cac agt tta cgt gag aag ttg | 531 |
| | Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu | |
| | 160 165 170 | |
| 15 | gct gaa atg gaa aca ttt aga gcc atc tta tgt aga caa gtt gac acg | 579 |
| | Ala Glu Met Glu Thr Phe Arg Ala Ile Leu Cys Arg Gln Val Asp Thr | |
| | 175 180 185 190 | |
| 20 | cta cag aag tac ttt gat gcc tgt gct gat gct gtc tct aag gat gaa | 627 |
| | Leu Gln Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu | |
| | 195 200 205 | |
| 25 | ctt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct aca | 675 |
| | Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr | |
| | 210 215 220 | |
| 30 | acg cgt tct gat ggt gac ttc ttg cat agt acc aac ggc aat aaa gaa | 723 |
| | Thr Arg Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu | |
| | 225 230 235 | |
| 35 | aag tta ttt cca cat gtg aca cca aaa gga att aat ggt ata gac ttt | 771 |
| | Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe | |
| | 240 245 250 | |
| 40 | aaa ggg gaa gcg ata act ttt aaa gca act act gct gga atc ctt gca | 819 |
| | Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala | |
| | 255 260 265 270 | |
| 45 | aca ctt tct cat tgt att gaa cta atg gtt aaa cgt gag gac agc tgg | 867 |
| | Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp | |
| | 275 280 285 | |
| 50 | cag aag aga ctg gat aag gaa act gag aag aaa aga aga aca gag gaa | 915 |
| | Gln Lys Arg Leu Asp Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu Glu | |
| | 290 295 300 | |
| 55 | gca tat aaa aat gca atg aca gaa ctt aag aaa aaa tcc cac ttt gga | 963 |
| | Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe Gly | |
| | 305 310 315 | |
| 60 | gga cca gat tat gaa gaa ggc cct aac agt ctg att aat gaa gaa gag | 1011 |
| | Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu Glu | |
| | 320 325 330 | |
| 65 | ttc ttt gat gct gtt gaa gct gct ctt gac aga caa gat aaa ata gaa | 1059 |
| | Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile Glu | |
| | 335 340 345 350 | |
| 70 | gaa cag tca cag agt gaa aag gtg aga tta cat tgg cct aca tcc ttg | 1107 |
| | Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser Leu | |
| | 355 360 365 | |
| 75 | ccc tct gga gat gcc ttt tct tct gtg ggg aca cat aga ttt gtc caa | 1155 |

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| | Pro | Ser | Gly | Asp | Ala | Phe | Ser | Ser | Val | Gly | Thr | His | Arg | Phe | Val | Gln | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| | | | | 370 | | | | | 375 | | | | | 380 | | | |
| 5 | aag | ccc | tat | agt | cgc | tct | tcc | tcc | atg | tct | tcc | att | gat | cta | gtc | agt | 1203 |
| | Lys | Pro | Tyr | Ser | Arg | Ser | Ser | Ser | Met | Ser | Ser | Ile | Asp | Leu | Val | Ser | |
| | | | 385 | | | | | 390 | | | | | 395 | | | | |
| 10 | gcc | tct | gat | gat | gtt | cac | aga | ttc | agc | tcc | cag | gtt | gaa | gag | atg | gtg | 1251 |
| | Ala | Ser | Asp | Asp | Val | His | Arg | Phe | Ser | Ser | Gln | Val | Glu | Glu | Met | Val | |
| | | | 400 | | | | 405 | | | | | 410 | | | | | |
| 15 | cag | aac | cac | atg | act | tac | tca | tta | cag | gat | gta | ggc | gga | gat | gcc | aat | 1299 |
| | Gln | Asn | His | Met | Thr | Tyr | Ser | Leu | Gln | Asp | Val | Gly | Gly | Asp | Ala | Asn | |
| | 415 | | | | 420 | | | | | 425 | | | | | | 430 | |
| 20 | tgg | cag | ttg | gtt | gta | gaa | gaa | gga | gaa | atg | aag | gta | tac | aga | aga | gaa | 1347 |
| | Trp | Gln | Leu | Val | Val | Glu | Glu | Gly | Glu | Met | Lys | Val | Tyr | Arg | Arg | Glu | |
| | | | | 435 | | | | | 440 | | | | | | 445 | | |
| 25 | gta | gaa | gaa | aat | ggg | att | gtt | ctg | gat | cct | tta | aaa | gct | acc | cat | gca | 1395 |
| | Val | Glu | Glu | Asn | Gly | Ile | Val | Leu | Asp | Pro | Leu | Lys | Ala | Thr | His | Ala | |
| | | | | 450 | | | | | 455 | | | | | | 460 | | |
| 30 | gtt | aaa | ggc | gtc | aca | gga | cat | gaa | gtc | tgc | aat | tat | ttc | tgg | aat | gtt | 1443 |
| | Val | Lys | Gly | Val | Thr | Gly | His | Glu | Val | Cys | Asn | Tyr | Phe | Trp | Asn | Val | |
| | | | 465 | | | | 470 | | | | | | 475 | | | | |
| 35 | gac | gtt | cgc | aat | gac | tgg | gaa | aca | act | ata | gaa | aac | ttt | cat | gtg | gtg | 1491 |
| | Asp | Val | Arg | Asn | Asp | Trp | Glu | Thr | Thr | Ile | Glu | Asn | Phe | His | Val | Val | |
| | | | 480 | | | | 485 | | | | | 490 | | | | | |
| 40 | gaa | aca | tta | gct | gat | aat | gca | atc | atc | att | tat | caa | aca | cac | aag | agg | 1539 |
| | Glu | Thr | Leu | Ala | Asp | Asn | Ala | Ile | Ile | Ile | Tyr | Gln | Thr | His | Lys | Arg | |
| | 495 | | | | | 500 | | | | | 505 | | | | | 510 | |
| 45 | gtg | tgg | cct | gct | tct | cag | cga | gac | gta | tta | tat | ctt | tct | gtc | att | cga | 1587 |
| | Val | Trp | Pro | Ala | Ser | Gln | Arg | Asp | Val | Leu | Tyr | Leu | Ser | Val | Ile | Arg | |
| | | | | 515 | | | | | 520 | | | | | | 525 | | |
| 50 | aag | ata | cca | gcc | ttg | act | gaa | aat | gac | cct | gaa | act | tgg | ata | gtt | tgt | 1635 |
| | Lys | Ile | Pro | Ala | Leu | Thr | Glu | Asn | Asp | Pro | Glu | Thr | Trp | Ile | Val | Cys | |
| | | | | 530 | | | | | 535 | | | | | 540 | | | |
| 55 | aat | tct | tct | gtg | gat | cat | gac | agt | gct | cct | cta | aac | aac | cga | tgt | gtc | 1683 |
| | Asn | Phe | Ser | Val | Asp | His | Asp | Ser | Ala | Pro | Leu | Asn | Asn | Arg | Cys | Val | |
| | | | | 545 | | | | 550 | | | | | 555 | | | | |
| 60 | cgt | gcc | aaa | ata | aat | gtt | gct | atg | att | tgt | caa | acc | ttg | gta | agc | cca | 1731 |
| | Arg | Ala | Lys | Ile | Asn | Val | Ala | Met | Ile | Cys | Gln | Thr | Leu | Val | Ser | Pro | |
| | | | | 560 | | | 565 | | | | | 570 | | | | | |
| 65 | cca | gag | gga | aac | cag | gaa | att | agc | agg | gac | aac | att | cta | tgc | aag | att | 1779 |
| | Pro | Glu | Gly | Asn | Gln | Glu | Ile | Ser | Arg | Asp | Asn | Ile | Leu | Cys | Lys | Ile | |
| | | | | 575 | | 580 | | | | | 585 | | | | 590 | | |
| 70 | aca | tat | gta | gct | aat | gtg | aac | cct | gga | gga | tgg | gca | cca | gcc | tca | gtg | 1827 |
| | Thr | Tyr | Val | Ala | Asn | Val | Asn | Pro | Gly | Gly | Trp | Ala | Pro | Ala | Ser | Val | |
| | | | | 595 | | | | | 600 | | | | | 605 | | | |
| 75 | tta | agg | gca | gtg | gca | aag | cga | gag | tat | cct | aaa | ttt | cta | aaa | cgt | ttt | 1875 |
| | Leu | Arg | Ala | Val | Ala | Lys | Arg | Glu | Tyr | Pro | Lys | Phe | Leu | Lys | Arg | Phe | |

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610 615 620
5 act tct tac gtc caa gaa aaa act gca gga aag cct att ttg ttc 1920
Thr Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
625 630 635
tagtattaac aggtactaga agatatgttt tatctttttt taactttatt tgactaatat 1980
10 gactgtcaat actaaaattt agttgttgaa agtatttact atgttttttc cggaattc 2038

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<211> 637
<212> PRT
<213> Artificial Sequence
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Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met Ser Asp
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5 Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu
 20 25 30

Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
 35 40 45

10 Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
 50 55 60

Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
 65 70 75 80

15 Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
 85 90 95

Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
 100 105 110

20 Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
 115 120 125

Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
 130 135 140

25 Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala Thr Ser
 145 150 155 160

30 Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu
 165 170 175

Met Glu Thr Phe Arg Ala Ile Leu Cys Arg Gln Val Asp Thr Leu Gln
 180 185 190

35 Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln
 195 200 205

Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg
 210 215 220

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Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu
 225 230 235 240

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 10 aattagctgg gtgcagtacc acacacttgt agtcccagct acccaggagg ctgggcaaaa 12360
 gattccttga gccagggagg tcaaggctgc aatgagccgc gtttgtgcca ctgcactcta 12420
 gcctgggtca caaagtgaga ccctgtgtga gatatatata tatatatata tatatatata 12480
 15 ta 12482

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30 Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr Thr Arg
 1 5 10 15
 Gly Phe Val Phe Thr
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45 Lys Gly Lys Arg Gly Asp Ala Gly Ser Pro Ala Thr Trp Thr Thr Arg
 1 5 10 15
 Gly Phe Val Phe Thr
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50 <210> 28
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ccgagcccga cgagttccag ctctgattat ccgacatctt gtcacgctcg 50

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cgggatccgc tagctaagcc aggcaaggat gg 32

<210> 31
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Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 33
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EP 1 144 650 B1

<400> 33

Pro Arg Ser Ala Arg Cys Gln Ala Arg Arg Arg Arg Gly Gly Arg Thr
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Ser Ser

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<210> 34

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<212> DNA

<213> Artificial Sequence

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<210> 35

<211> 18

<212> DNA

<213> Artificial Sequence

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18

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<210> 36

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<212> DNA

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<220>

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18

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<210> 37

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<212> DNA

<213> Artificial Sequence

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tagaagaaca gtcacagagt gaaaagg

27

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<210> 38

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<212> DNA

<213> Artificial Sequence

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Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu Phe Val Lys Val Leu
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Arg Ser Pro

<210> 44
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 Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
 1 5 10 15

acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca gca att cct 96
 Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
 20 25 30

tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt tct ttt ctg 144
 Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
 35 40 45

ttt gta caa gga aat caa cga gcc cac gga caa gac ctt gga act ctt 192
 Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Gly Thr Leu
 50 55 60

ggc agc tgc ctg cag cga ttt acc aca atg cca ttc tta ttc tgc aat 240
 Gly Ser Cys Leu Gln Arg Phe Thr Thr Met Pro Phe Leu Phe Cys Asn
 65 70 75 80

gtc aat gat gta tgt aat ttt gca tct cga aat gat tat tca tac tgg 288
 Val Asn Asp Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp
 85 90 95

ctg tca aca cca gct ctg atg cca atg aac atg gct ccc att act ggc 336
 Leu Ser Thr Pro Ala Leu Met Pro Met Asn Met Ala Pro Ile Thr Gly

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| | 100 | | 105 | | 110 | |
|----|---|-----|-----|-----|-----|-----|
| 5 | aga gcc ctt gag cct tat ata agc aga tgc act gtt tgt gaa ggt cct | | | | | 384 |
| | Arg Ala Leu Glu Pro Tyr Ile Ser Arg Cys Thr Val Cys Glu Gly Pro | | | | | |
| | 115 | | 120 | | 125 | |
| 10 | gcg atc gcc ata gcc gtt cac agc caa acc act gac att cct cca tgt | | | | | 432 |
| | Ala Ile Ala Ile Ala Val His Ser Gln Thr Thr Asp Ile Pro Pro Cys | | | | | |
| | 130 | | 135 | | 140 | |
| 15 | cct cac ggc tgg att tct ctc tgg aaa gga ttt tca ttc atc atg aaa | | | | | 480 |
| | Pro His Gly Trp Ile Ser Leu Trp Lys Gly Phe Ser Phe Ile Met Lys | | | | | |
| | 145 | | 150 | | 155 | 160 |
| 20 | gcc tat tcc atc aac tgt gaa agc tgg gga att aga aaa aat aat aag | | | | | 528 |
| | Ala Tyr Ser Ile Asn Cys Glu Ser Trp Gly Ile Arg Lys Asn Asn Lys | | | | | |
| | | 165 | | 170 | | 175 |
| 25 | tcg ctg tca ggt gtg cat gaa gaa aag aca ctg aag cta aaa aag aca | | | | | 576 |
| | Ser Leu Ser Gly Val His Glu Glu Lys Thr Leu Lys Leu Lys Lys Thr | | | | | |
| | | 180 | | 185 | | 190 |
| 30 | gca gaa ctg cta ttt ttc atc cta aag aac aaa gta atg aca gaa cat | | | | | 624 |
| | Ala Glu Leu Leu Phe Phe Ile Leu Lys Asn Lys Val Met Thr Glu His | | | | | |
| | | 195 | | 200 | | 205 |
| 35 | gct gtt att taggtat ttt tctttaacca aacaatattg ctccatgatg | | | | | 673 |
| | Ala Val Ile | | | | | |
| | 210 | | | | | |
| 40 | acttagtaca aa | | | | | 685 |

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<211> 211

35 <212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: GPDV

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Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
 1 5 10 15
 Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
 5 20 25 30
 Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
 35 40 45
 Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Gly Thr Leu
 10 50 55 60
 Gly Ser Cys Leu Gln Arg Phe Thr Thr Met Pro Phe Leu Phe Cys Asn
 65 70 75 80
 Val Asn Asp Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp
 15 85 90 95
 Leu Ser Thr Pro Ala Leu Met Pro Met Asn Met Ala Pro Ile Thr Gly
 20
 100 105 110
 Arg Ala Leu Glu Pro Tyr Ile Ser Arg Cys Thr Val Cys Glu Gly Pro
 25 115 120 125
 Ala Ile Ala Ile Ala Val His Ser Gln Thr Thr Asp Ile Pro Pro Cys
 130 135 140
 Pro His Gly Trp Ile Ser Leu Trp Lys Gly Phe Ser Phe Ile Met Lys
 30 145 150 155 160
 Ala Tyr Ser Ile Asn Cys Glu Ser Trp Gly Ile Arg Lys Asn Asn Lys
 165 170 175
 Ser Leu Ser Gly Val His Glu Glu Lys Thr Leu Lys Leu Lys Lys Thr
 35 180 185 190
 Ala Glu Leu Leu Phe Phe Ile Leu Lys Asn Lys Val Met Thr Glu His
 40 195 200 205
 Ala Val Ile
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 <210> 47
 <211> 680
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 55 <400> 47

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1 ggt ttg aaa gga aaa cgt gga gac agt gga tca cct gca acc tgg aca 48
 Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
 1 5 10 15

5 acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca gca att cct 96
 Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
 20 25 30

10 tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt tct ttt ctt 144
 Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
 35 40 45

15 ttt gta caa gga aat caa cga gcc cac gga caa gac ctt gat gca ctg 192
 Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu
 50 55 60

20 ttt gtg aag gtc ctg cga tcg cca tagcgcgttca cagccaaacc actgacattc 246
 Phe Val Lys Val Leu Arg Ser Pro
 65 70

ctccatgtcc tcacggctgg atttctctct ggaaaggatt ttcattcadc atgttcacaa 306
 gtgcagggttc tgagggcacc gggcaagcac tggcctcccc tggctcctgc ctggaagaat 366

25 tccgagccag cccatttcta gaatgtcatg gaagaggaac gtgcaactac tattcaaatt 426
 cctacagttt ctggctggct tcattaaacc cagaaagaat gttcagaaag cctattccat 486

30 caactgtgaa agctggggaa ttagaaaaaa taataagtcg ctgtcagggtg tgcatagaaga 546
 aaagacactg aagctaaaaa agacagcaga actgctattt ttcatacctaa agaacaaagt 606
 aatgacagaa catgctgtta tttaggtatt tttctttaac caaacaatat tgctccatga 666

35 tgacttagta caaa 680

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40 <211> 72

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: GPDIII

45 <400> 48

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EP 1 144 650 B1

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 1 5 10 15
 5 Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
 20 25 30
 Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
 35 40 45
 10 Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu
 50 55 60
 Phe Val Lys Val Leu Arg Ser Pro
 65 70

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<210> 49
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 25 <222> (1)..(207)
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30 ggt ttg aaa gga aaa cgt gga gac agt gga tca cct gca acc tgg aca 48
 Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
 1 5 10 15
 acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca gca att cct 96
 Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
 20 25 30
 35 tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt tct ttt ctt 144
 Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu

40

35 40 45

45 ttt gta caa gga aat caa cga gcc cac gga caa gac ctt gaa agc cta 192
 Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Glu Ser Leu
 50 55 60

50 ttc cat caa ctg tga aagctgggga attagaaaaa ataataagtc gctgtcaggt 247
 Phe His Gln Leu
 65

gtgcatgaag aaaagacact gaagctaaaa aagacagcag aactgctatt tttcatccta 307

aagaacaaag taatgacaga acatgctgtt atttaggtat ttttctttaa ccaaacaata 367

55 ttgctccatg atgacttagt acaaaa 392

<210> 50

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<211> 68
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5 <223> Description of Artificial Sequence: GPDIII-IV-V
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 1 5 10 15
 Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
 20 25 30
 15 Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
 35 40 45
 Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Glu Ser Leu
 50 55 60
 20 Phe His Gln Leu
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<210> 51
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25 <223> Description of Artificial Sequence: GPDIII-V
 30 <220>
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 <222> (1)..(216)
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 Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
 1 5 10 15
 40 acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca gca att cct 96
 Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro

45

50

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                20                25                30
5      tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt tct ttt ctt 144
      Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
                35                40                45

10     ttt gta caa gga aat caa cga gcc cac gga caa gac ctt gat gca ctg 192
      Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu
                50                55                60

15     ttt gtg aag gtc ctg cga tcg cca tagccgttca cagccaaacc actgacattc 246
      Phe Val Lys Val Leu Arg Ser Pro
                65                70

20     ctccatgtcc tcacggctgg atttctctct ggaaaggatt ttcattcattc atgaaagcct 306
      attccatcaa ctgtgaaagc tggggaatta gaaaaaataa taagtcgctg tcaggtgtgc 366
      atgaagaaaa gacactgaag ctaaaaaaga cagcagaact gctatTTTTc atcctaaaga 426
      acaaagtaat gacagaacat gctgttattt aggtatTTTT cttaaccaa acaatattgc 486
      tccatgatga cttagtacaa a 507

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<210> 52
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<212> PRT
30 <213> Artificial Sequence
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<400> 52

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      Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
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40     Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
                20          25          30
      Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
                35          40          45
45     Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu
                50          55          60
      Phe Val Lys Val Leu Arg Ser Pro
        65          70

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<210> 53
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<222> (37)..(627)

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1 5

10 ccc tcc cag agg cac gga tcc aag tac ctg gcc aca gca agt acc atg 102
Pro Ser Gln Arg His Gly Ser Lys Tyr Leu Ala Thr Ala Ser Thr Met
10 15 20

15 gac cat gcc agg cat ggc ttc ctc cca agg cac aga gac acg ggc atc 150
Asp His Ala Arg His Gly Phe Leu Pro Arg His Arg Asp Thr Gly Ile
25 30 35

20 ctt gac tcc atc ggg cgc ttc ttt ggc ggt gac agg ggt gcg cca aag 198
Leu Asp Ser Ile Gly Arg Phe Phe Gly Gly Asp Arg Gly Ala Pro Lys
40 45 50

25 cgg ggc tct ggc aag gta ccc tgg cta aag ccg gcc cgg agc cct ctg 246
Arg Gly Ser Gly Lys Val Pro Trp Leu Lys Pro Gly Arg Ser Pro Leu
55 60 65 70

30 ccc tct cat gcc cgc agc cag cct ggg ctg tgc aac atg tac aag gac 294
Pro Ser His Ala Arg Ser Gln Pro Gly Leu Cys Asn Met Tyr Lys Asp
75 80 85

35 tca cac cac ccg gca aga act gct cac tat ggc tcc ctg ccc cag aag 342
Ser His His Pro Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys
90 95 100

40 tca cac ggc cgg acc caa gat gaa aac ccc gta gtc cac ttc ttc aag 390
Ser His Gly Arg Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys
105 110 115

45 aac att gtg acg cct cgc aca cca ccc ccg tcg cag gga aag ggg aga 438
Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly Arg
120 125 130

50 gga ctg tcc ctg agc aga ttt agc tgg ggg gcc gaa ggc cag aga cca 486
Gly Leu Ser Leu Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln Arg Pro
135 140 145 150

55 gga ttt ggc tac gga ggc aga gcg tcc gac tat aaa tcg gct cac aag 534
Gly Phe Gly Tyr Gly Gly Arg Ala Ser Asp Tyr Lys Ser Ala His Lys
155 160 165

60 gga ttc aag gga gtc gat gcc cag ggc acg ctt tcc aaa att ttt aag 582
Gly Phe Lys Gly Val Asp Ala Gln Gly Thr Leu Ser Lys Ile Phe Lys
170 175 180

65 ctg gga gga aga gat agt cgc tct gga tca ccc atg gct aga cgc 627
Leu Gly Gly Arg Asp Ser Arg Ser Gly Ser Pro Met Ala Arg Arg
185 190 195

70 tgaaaaccca cctggttcg gaatcctgtc ct 659

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<210> 54

<211> 197

<212> PRT

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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: HMBP-21

<400> 54

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Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His Gly Ser Lys Tyr Leu
1 5 10 15

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Ala Thr Ala Ser Thr Met Asp His Ala Arg His Gly Phe Leu Pro Arg
20 25 30

His Arg Asp Thr Gly Ile Leu Asp Ser Ile Gly Arg Phe Phe Gly Gly
35 40 45

15

Asp Arg Gly Ala Pro Lys Arg Gly Ser Gly Lys Val Pro Trp Leu Lys
50 55 60

Pro Gly Arg Ser Pro Leu Pro Ser His Ala Arg Ser Gln Pro Gly Leu
65 70 75 80

20

Cys Asn Met Tyr Lys Asp Ser His His Pro Ala Arg Thr Ala His Tyr
85 90 95

Gly Ser Leu Pro Gln Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro
100 105 110

25

Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro
115 120 125

Ser Gln Gly Lys Gly Arg Gly Leu Ser Leu Ser Arg Phe Ser Trp Gly
130 135 140

30

Ala Glu Gly Gln Arg Pro Gly Phe Gly Tyr Gly Arg Ala Ser Asp
145 150 155 160

Tyr Lys Ser Ala His Lys Gly Phe Lys Gly Val Asp Ala Gln Gly Thr
165 170 175

35

Leu Ser Lys Ile Phe Lys Leu Gly Gly Arg Asp Ser Arg Ser Gly Ser
180 185 190

40

Pro Met Ala Arg Arg
195

45 **Claims**

1. An isolated nucleic acid sequence encoding a functional protein kinase comprising sequence sharing at least 70% identity with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25.
2. An isolated nucleic acid sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25.
3. An isolated nucleic acid comprising a sequence that encodes a polypeptide selected from the group consisting of GPBP, GPBP Δ 26, and GPBPp₁, or fractionally equivalent fragments thereof encoding a functional protein kinase, wherein GPBP is characterized by SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, GPBP Δ 26 is characterized

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by SEQ ID NO:8, SEQ ID NO:10 and SEQ ID NO:12, and GPBPpep1 is **characterized by** SEQ ID NO:14.

4. An isolated nucleic acid sequence encoding a functional protein kinase comprising a sequence that encodes a protein sequence sharing at least 70% identify with a protein sequence selected from the group consisting of SEQ ID NQ:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16; SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:24
5. An isolated nucleic acid sequence comprising a sequence that encodes a protein sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:24.
6. A recombinant expression vector comprising the isolated nucleic acid sequence of any one of claims 1-5.
7. A recombinant expression vector comprising an isolated nucleic acici sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25; or functionally equivalent fragments thereof.
8. A host cell transfected with the recombinant expression vector of claim 6 or 7.
9. A substantially purified polypeptide of a functional kinase, comprising an amino acid sequence sharing at least 70% identify with a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or functionally equivalent peptide fragments thereof.
10. A substantially purified polypeptide, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or functionally equivalent peptide fragments thereof encoding a functional protein kinase.
11. A substantially purified protein comprising a polypeptide selected from the group consisting of GPBP, GPBP Δ 25, and GPBPpep1, or functionally equivalent peptide fragments thereof encoding a functional protein kinase, wherein GPBP is **characterized by** SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:5, GPBP Δ 26 is **characterized by** SEQ ID NO:8, SEQ ID NO:10 and SEQ ID NO:12, and wherein GPBPpep1 is ctaraderized by SEQ ID NO:14.
12. An antibody that selectively binds to the substantially purified protein or polypeptide of any one of claims 9-11.
13. The antibody of claim 12, wherein the antibody is a polyclonal antibody.
14. The antibody of claim 12, wherein the antibody is a monoclonal antibody.
15. A method for detecting the presence of a protein that shares at least 70% identify with a protein selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, comprising
 - a) contacting a protein sample to be screened with the antibody of any one of claims 12-14 under conditions that promote antibody-antigen complex formation; and
 - b) detecting the formation of antibody-antigen complexes, wherein the presence of the antibody-antigen complex indicates the presence of a protein that is substantially similar to a protein selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24.
16. The method of claim 15, wherein detecting comprises s method selected from the group consisting of immunolocalization, immunofluorescence analysis, Western blot analysis, ELISAs, and nucleic acid expression library screening.
17. A method for detecting in a sample a sequence that shares at least 70% identity with a maleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9; SEQ ID NO:11,

SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:25, comprising contacting the sample with the isolated nucleic acid of any one of claims 1-5, or functionally equivalent fragments thereof encoding a functional protein kinase and detecting complex formation, wherein complex formation indicates the presence in the sample of the sequence that shares at least 70% identity with a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:23.

18. The method of claim 17, wherein the detecting is carried out by a method selected from the group consisting of hybridization, reverse transcription, PCR, coupled reverse transcription-PCR, Northern blotting, Southern blotting, and DNA library screening.

19. A method for detecting an autoimmune condition in a patient, comprising detecting altered GPBP RNA or protein expression in a tissue or body fluid sample from the patient compared to a control tissue or body fluid sample in which no autoimmune condition is present, wherein an alteration in GPBP RNA or protein expression relative to the control indicates the presence of an autoimmune condition,

20. A method for detecting cells undergoing apoptosis or cancer transformation in a tissue or body fluid sample, comprising detecting altered GPBP RNA or protein expression in a tissue or body fluid sample from the patient compared to a normal control tissue or body fluid sample, wherein an alteration in GPBP RNA or protein expression relative to the control indicates the presence of cells undergoing apoptosis or cancer transformation, wherein GPBP is **characterized by** SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6.

21. A use of a GPBP antibody, or an antisense oligomer for the preparation of a pharmaceutical composition for treating a patient with an autoimmune disorder, wherein the expression or activity of GPBP, GPBP Δ 26, or a protein comprising a polypeptide sharing at least for identify with GPBP_{pep1} is modified in the patient with the autoimmune disorder, wherein GPBP is **characterized by** SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, GPBP Δ 26 is **characterized by** SEQ ID NO.8, SEQ ID NO:10 and SEQ ID NO:12 and wherein GPBP_{pep1} is **characterized by** SEQ ID NO:14.

22. A use of a GPBP antibody, or an antisense oligomer for the preparation of a pharmaceutical composition for treating a patient with a tumor, wherein the expression or activity of GPBP, GPBP Δ 26, or a protein comprising a polypeptide sharing at least 70% for identify with GPBP_{pep1} is modified in the patient with the tumor, wherein GPBP is **characterized by** SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, GPBP Δ 26 is **characterized by** SEQ ID NO.8, SEQ ID NO:10 and SEQ ID NO:12, and wherein GPBP_{pep1} is **characterized by** SEQ ID NO:14.

23. A use of a GPBP antibody, or an antisense oligomer for the preparation of a pharmaceutical composition for preventing cell apoptosis, wherein the expression or activity of GPBP, GPBP Δ 26, or a protein comprising a polypeptide sharing at least of identify with to GPBP _{pep1} is modified in the cell, wherein GPBP is **characterized by** SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, GPBP Δ 26 is **characterized by** SEQ ID NO.8, SEQ ID NO:10 and SEQ ID NO:12, and wherein GPBP_{pep1} is **characterized by** SEQ ID NO:14.

Patentansprüche

1. Isolierte Nukleinsäuresequenz, die für eine funktionelle Proteinkinase kodiert, umfassend eine Sequenz, die eine Identität von mindestens 70% mit einer Nukleinsäuresequenz ausgewählt aus der Gruppe bestehend aus SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21; SEQ ID NO:23 und SEQ ID NO:25 aufweist.

2. Isolierte Nukleinsäuresequenz, umfassend eine Nukleinsäuresequenz, ausgewählt aus der Gruppe bestehend aus SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 und SEQ ID NO:25.

3. Isolierte Nukleinsäure, umfassend eine Sequenz, die für ein Polypeptid kodiert, ausgewählt aus der Gruppe bestehend aus GPBP, GPBP Δ 26 und GPBP_{pep1}, oder funktionell äquivalente Fragmente davon, die für eine funktionelle Proteinkinase kodieren, wobei GPBP **gekennzeichnet ist durch** SEQ ID NO:2, SEQ ID NO:4 und SEQ ID NO:6, GPBP Δ 26 **gekennzeichnet ist durch** SEQ ID NO:8, SEQ ID NO:10 und SEQ ID NO:12 und GPBP_{pep1} **gekennzeichnet ist durch** SEQ ID NO:14.

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4. Isolierte Nukleinsäuresequenz, die für eine funktionelle Proteinkinase kodiert, umfassend eine Sequenz, die für eine Proteinsequenz kodiert, die eine Identität von mindestens 70% mit einer Proteinsequenz ausgewählt aus der Gruppe bestehend aus SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 und SEQ ID NO:24 aufweist.
5
5. Isolierte Nukleinsäuresequenz, umfassend eine Sequenz, die für eine Proteinsequenz kodiert, ausgewählt aus der Gruppe bestehend aus SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 und SEQ ID NO:24.
- 10 6. Rekombinanter Expressionsvektor, umfassend die isolierte Nukleinsäuresequenz nach einem der Ansprüche 1 bis 5.
7. Rekombinanter Expressionsvektor, umfassend eine isolierte Nukleinsäuresequenz, die eine Nukleinsäuresequenz ausgewählt aus der Gruppe bestehend aus SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 und SEQ ID NO:25 oder funktionell äquivalenten Fragmenten davon, umfasst.
15
8. Wirtszelle, die mit dem rekombinanten Expressionsvektor nach Anspruch 6 oder 7 transfiziert ist.
9. Substanziell gereinigtes Polypeptid einer funktionellen Kinase, umfassend eine Aminosäuresequenz, die eine Identität von mindestens 70% mit einer Sequenz ausgewählt aus der Gruppe bestehend aus SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24 oder funktionell äquivalenten Peptidfragmenten davon, aufweist.
20
10. Substanziell gereinigtes Polypeptid, umfassend eine Aminosäuresequenz, ausgewählt aus der Gruppe bestehend aus SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24 oder funktionell äquivalenten Peptidfragmenten davon, die für eine funktionelle Proteinkinase kodieren.
25
11. Substanziell gereinigtes Protein, umfassend ein Polypeptid, ausgewählt aus der Gruppe bestehend aus GPBP, GPBP Δ 26 und GPBP Δ 26 pep1 oder funktionell äquivalenten Peptidfragmenten davon, die für eine funktionelle Proteinkinase kodieren, wobei GPBP **gekennzeichnet ist durch** SEQ ID NO:2, SEQ ID NO:4 und SEQ ID NO:6, GPBP Δ 26 **gekennzeichnet ist durch** SEQ ID NO:8, SEQ ID NO:10 und SEQ ID NO:12 und GPBP Δ 26 pep1 **gekennzeichnet ist durch** SEQ ID NO:14.
30
12. Antikörper, der selektiv an ein substanziell gereinigtes Protein oder Polypeptid nach einem der Ansprüche 9 bis 11 bindet.
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13. Antikörper nach Anspruch 12, wobei der Antikörper ein polyklonaler Antikörper ist.
- 40 14. Antikörper nach Anspruch 12, wobei der Antikörper ein monoklonaler Antikörper ist.
15. Verfahren zur Detektion des Vorliegens eines Proteins, das eine Identität von mindestens 70% mit einem Protein aufweist, ausgewählt aus der Gruppe bestehend aus SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, umfassend
45
 - a) In-Kontakt-Bringen einer zu durchmusternden Proteinprobe mit dem Antikörper nach einem der Ansprüche 12 bis 14 unter Bedingungen, welche eine Antikörper-Antigenkomplex-Bildung fördern; und
 - b) Nachweis der Bildung von Antikörper-Antigenkomplexen, wobei das Vorliegen eines Antikörper-Antigenkomplexes auf das Vorliegen eines Proteins hinweist, das substanziell ähnlich einem Protein ist, das aus der Gruppe bestehend aus SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24 ausgewählt ist.
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16. Verfahren nach Anspruch 15, wobei die Detektion ein Verfahren ausgewählt aus der Gruppe bestehend aus Immunolokalisation, Immunofluoreszenzanalyse, Western Blot-Analyse, ELISAs und Screening einer Nukleinsäureexpressionsbibliothek umfasst.
55
17. Verfahren zur Detektion einer Sequenz in einer Probe, die eine Identität von mindestens 70% mit einer Nukleinsäure

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ausgewählt aus der Gruppe bestehend aus SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 oder SEQ ID NO:25 aufweist, umfassend das In-Kontakt-Bringen der Probe mit der isolierten Nukleinsäure nach einem der Ansprüche 1 bis 5, oder funktionell äquivalenten Fragmenten davon, die für eine funktionelle Proteinkinase kodieren und das Detektieren der Komplexbildung, wobei eine Komplexbildung auf das Vorliegen der Sequenz in der Probe hinweist, die eine Identität von mindestens 70% mit einer Nukleinsäure aufweist, die aus der Gruppe bestehend aus SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 oder SEQ ID NO:25 ausgewählt ist.

18. Verfahren nach Anspruch 17, wobei die Detektion durch ein Verfahren durchgeführt wird, ausgewählt aus der Gruppe bestehend aus Hybridisierung, reverser Transkription, PCR, gekoppelte reverse Transkription-PCR, Northern Blotting, Southern Blotting und DNA-Bibliothek-Screening.

19. Verfahren zur Detektion eines Autoimmunzustandes in einem Patienten, umfassend das Detektieren einer geänderten GPBP-RNA- oder Protein-Expression in einer Gewebe- oder Körperflüssigkeitsprobe von dem Patienten im Vergleich zu einer Kontrollgewebe- oder Körperflüssigkeitsprobe, bei der kein Autoimmunzustand vorliegt, wobei eine Änderung bei der GPBP-RNA- oder Protein-Expression relativ zu der Kontrolle auf das Vorliegen eines Autoimmunzustandes hinweist, worin GPBP **gekennzeichnet ist durch** SEQ ID NO:2, SEQ ID NO:4 und SEQ ID NO:6.

20. Verfahren zur Detektion von Zellen, die eine Apoptose oder Krebsmetastase durchmachen in einer Gewebe- oder Körperflüssigkeitsprobe, umfassend das Detektieren einer geänderten GPBP-RNA- oder Protein-Expression in einer Gewebe- oder Körperflüssigkeitsprobe von dem Patienten im Vergleich zu einer normalen Kontrollgewebe- oder Körperflüssigkeitsprobe, wobei eine Änderung bei der GPBP-RNA- oder Protein-Expression relativ zu der Kontrolle auf das Vorliegen von Zellen hinweist, die eine Apoptose oder Krebsmetastase durchmachen, worin GPBP **gekennzeichnet ist durch** SEQ ID NO:2, SEQ ID NO:4 und SEQ ID NO:6.

21. Verwendung eines GPBP-Antikörpers oder eines Antisinn-Oligomers für die Herstellung einer pharmazeutischen Zusammensetzung zur Behandlung eines Patienten mit einer Autoimmunerkrankung, wobei die Expression oder Aktivität von GPBP, GPBP Δ 26 oder ein Protein, das ein Polypeptid umfasst, das eine Identität von mindestens 70% mit GPBP_{pep1} aufweist, in dem Patienten mit der Autoimmunerkrankung modifiziert ist, worin GPBP **gekennzeichnet ist durch** SEQ ID NO:2, SEQ ID NO:4 und SEQ ID NO:6, GPBP Δ 26 **gekennzeichnet ist durch** SEQ ID NO:8, SEQ ID NO:10 und SEQ ID NO:12, und worin GPBP_{prep1} **gekennzeichnet ist durch** SEQ ID NO:14.

22. Verwendung eines GPBP-Antikörpers oder eines Antisinn-Oligomers für die Herstellung einer pharmazeutischen Zusammensetzung zur Behandlung eines Patienten mit einem Tumor, wobei die Expression oder Aktivität von GPBP, GPBP Δ 26 oder ein Protein, das ein Polypeptid umfasst, das eine Identität von mindestens 70% mit GPBP_{pep1} aufweist, in dem Patienten mit dem Tumor modifiziert ist, worin GPBP **gekennzeichnet ist durch** SEQ ID NO:2, SEQ ID NO:4 und SEQ ID NO:6, GPBP Δ 26 **gekennzeichnet ist durch** SEQ ID NO:8, SEQ ID NO:10 und SEQ ID NO:12, und worin GPBP_{prep1} **gekennzeichnet ist durch** SEQ ID NO:14.

23. Verwendung eines GPBP-Antikörpers oder eines Antisinn-Oligomers für die Herstellung einer pharmazeutischen Zusammensetzung zur Vorbeugung von Zell-Apoptose, wobei die Expression oder Aktivität von GPBP, GPBP Δ 26 oder ein Protein, das ein Polypeptid umfasst, das eine Identität von mindestens 70% mit GPBP_{pep1} aufweist, in der Zelle modifiziert ist, worin GPBP **gekennzeichnet ist durch** SEQ ID NO:2, SEQ ID NO:4 und SEQ ID NO:6, GPBP Δ 26 **gekennzeichnet ist durch** SEQ ID NO:8, SEQ ID NO:10 und SEQ ID NO:12, und worin GPBP_{prep1} **gekennzeichnet ist durch** SEQ ID NO:14.

Revendications

1. Séquence d'acide nucléique isolée codant une protéine kinase fonctionnelle comprenant une séquence partageant une identité d'au moins 70 % avec une séquence d'acide nucléique choisie dans l'ensemble consistant en SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 et SEQ ID NO:25.

2. Séquence d'acide nucléique isolée comprenant une séquence d'acide nucléique choisie dans l'ensemble consistant en SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ

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ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 et SEQ ID NO:25.

- 5 3. Acide nucléique isolé comprenant une séquence qui code un polypeptide choisi dans l'ensemble consistant en GPBP, GPBP Δ 26 et GPBPpep1, ou leurs fragments fonctionnellement équivalents, GPBP étant **caractérisé par** SEQ ID NO:2, SEQ ID NO:4 et SEQ ID NO:6, GPBP Δ 26 étant **caractérisé par** SEQ ID NO:8, SEQ ID NO:10 et SEQ ID NO:12 et GPBPpep1 étant **caractérisé par** SEQ ID NO:14.
- 10 4. Séquence d'acide nucléique isolée codant une protéine kinase fonctionnelle comprenant une séquence qui code une séquence protéique partageant une identité d'au moins 70 % avec une séquence protéique choisie dans l'ensemble consistant en SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 et SEQ ID NO:24.
- 15 5. Séquence d'acide nucléique isolée comprenant une séquence qui code une séquence protéique choisie dans l'ensemble consistant en SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 et SEQ ID NO:24.
- 20 6. Vecteur d'expression recombinant comprenant la séquence d'acide nucléique isolée de l'une quelconque des revendications 1-5.
- 25 7. Vecteur d'expression recombinant comprenant une séquence d'acide nucléique isolée comprenant une séquence d'acide nucléique choisie dans l'ensemble consistant en SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 et SEQ ID NO:25, ou leurs fragments fonctionnellement équivalents.
- 30 8. Cellule hôte transfectée par le vecteur d'expression recombinant de la revendication 6 ou 7.
- 35 9. Polypeptide d'une kinase fonctionnelle sensiblement purifié, comprenant une séquence d'acides aminés partageant une identité d'au moins 70 % avec une séquence choisie dans l'ensemble consistant en SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 et SEQ ID NO:24 ou leurs fragments peptidiques fonctionnellement équivalents.
- 40 10. Polypeptide sensiblement purifié, comprenant une séquence d'acides aminés choisie dans l'ensemble consistant en SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 et SEQ ID NO:24 ou leurs fragments peptidiques fonctionnellement équivalents.
- 45 11. Protéine sensiblement purifiée, comprenant un polypeptide choisi dans l'ensemble consistant en GPBP, GPBP Δ 26 et GPBPpep1, ou leurs fragments peptidiques fonctionnellement équivalents, GPBP étant **caractérisé par** SEQ ID NO:2, SEQ ID NO:4 et SEQ ID NO:6, GPBP Δ 26 étant **caractérisé par** SEQ ID NO:8, SEQ ID NO:10 et SEQ ID NO:12 et GPBPpep1 étant **caractérisé par** SEQ ID NO:14.
- 50 12. Anticorps qui se lie spécifiquement à la protéine ou au polypeptide sensiblement purifié de l'une quelconque des revendications 9-11.
- 55 13. Anticorps de la revendication 12, lequel anticorps est un anticorps polyclonal.
14. Anticorps de la revendication 12, lequel anticorps est un anticorps monoclonal.
15. Procédé pour détecter la présence d'une protéine qui partage une identité d'au moins 70 % avec une protéine choisie dans l'ensemble consistant en SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 et SEQ ID NO:24, comprenant les étapes consistant
 - a) à mettre en contact un échantillon de protéines à cribler avec l'anticorps de l'une quelconque des revendications 12-14 dans des conditions qui favorisent la formation de complexes anticorps-antigène ; et
 - b) à détecter la formation de complexes anticorps-antigène, la présence d'un complexe anticorps-antigène indiquant la présence d'une protéine qui est sensiblement similaire à une protéine choisie dans l'ensemble consistant en SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ

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ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 et SEQ ID NO:24.

- 5
16. Procédé de la revendication 15, dans lequel la détection comprend un procédé choisi dans l'ensemble consistant en immunolocalisation, analyse d'immunofluorescence, analyse par transfert Western, tests ELISA et criblage d'une banque d'expression d'acide nucléique.
- 10
17. Procédé pour détecter dans un échantillon une séquence qui partage une identité d'au moins 70 % avec un acide nucléique choisi dans l'ensemble consistant en SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 ou SEQ ID NO:25, comprenant les étapes consistant à mettre en contact l'échantillon avec l'acide nucléique de l'une quelconque des revendications 1-5 ou l'un de ses fragments fonctionnellement équivalents codant une protéine kinase fonctionnelle et à détecter la formation de complexes, la formation de complexes indiquant la présence dans l'échantillon de la séquence qui partage une identité d'au moins 70 % avec un acide nucléique choisi dans l'ensemble consistant en SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 ou SEQ ID NO:25.
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18. Procédé de la revendication 17, dans lequel la détection est effectuée par un procédé choisi dans l'ensemble consistant en une hybridation, une transcription inverse, une PCR, une transcription inverse-PCR couplées, un transfert Northern, un transfert Southern, et le criblage d'une banque d'ADN.
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19. Procédé pour détecter une condition auto-immune chez un patient, comprenant la détection d'une expression d'ARN de GPBP ou de protéine GPBP altérée dans un échantillon de tissu ou de fluide corporel du patient comparé à un échantillon de tissu ou de fluide corporel témoin dans lequel aucune condition auto-immune n'est présente, l'altération de l'expression d'ARN de GPBP ou de protéine GPBP par rapport au témoin indiquant la présence d'une condition auto-immune, GPBP étant **caractérisé par** SEQ ID NO:2, SEQ ID NO:4 et SEQ ID NO:6.
- 25
20. Procédé pour détecter des cellules subissant une apoptose ou une transformation cancéreuse dans un échantillon de tissu ou de fluide corporel, comprenant la détection d'une expression d'ARN de GPBP ou de protéine GPBP altérée dans un échantillon de tissu ou de fluide corporel du patient comparé à un échantillon de tissu ou de fluide corporel témoin normal, l'altération de l'expression d'ARN de GPBP ou de protéine GPBP par rapport au témoin indiquant la présence de cellules subissant une apoptose ou une transformation cancéreuse, GPBP étant **caractérisé par** SEQ ID NO:2, SEQ ID NO:4 et SEQ ID NO:6.
- 30
21. Utilisation d'un anticorps contre GPBP ou d'un oligomère antisens pour la préparation d'une composition pharmaceutique pour le traitement d'un patient ayant une affection auto-immune, l'expression ou l'activité de GPBP, de GPBP Δ 26 ou d'une protéine comprenant un polypeptide partageant au moins 70 % d'identité avec GPBP_{pep1} étant modifiée chez le patient ayant l'affection auto-immune, GPBP étant **caractérisé par** SEQ ID NO:2, SEQ ID NO:4 et SEQ ID NO:6, GPBP Δ 26 étant **caractérisé par** SEQ ID NO:8, SEQ ID NO:10 et SEQ ID NO:12 et GPBP_{pep1} étant **caractérisé par** SEQ ID NO:14.
- 35
- 40
22. Utilisation d'un anticorps contre GPBP ou d'un oligomère antisens pour la préparation d'une composition pharmaceutique pour le traitement d'un patient ayant une tumeur, l'expression ou l'activité de GPBP, de GPBP Δ 26 ou d'une protéine comprenant un polypeptide partageant au moins 70 % d'identité avec GPBP_{pep1} étant modifiée chez le patient ayant la tumeur, GPBP étant **caractérisé par** SEQ ID NO:2, SEQ ID NO:4 et SEQ ID NO:6, GPBP Δ 26 étant **caractérisé par** SEQ ID NO:8, SEQ ID NO:10 et SEQ ID NO:12 et GPBP_{pep1} étant **caractérisé par** SEQ ID NO:14.
- 45
23. Utilisation d'un anticorps contre GPBP ou d'un oligomère antisens pour la préparation d'une composition pharmaceutique pour prévenir une apoptose cellulaire, l'expression ou l'activité de GPBP, de GPBP Δ 26 ou d'une protéine comprenant un polypeptide partageant au moins 70 % d'identité avec GPBP_{pep1} étant modifiée dans la cellule, GPBP étant **caractérisé par** SEQ ID NO:2, SEQ ID NO:4 et SEQ ID NO:6, GPBP Δ 26 étant **caractérisé par** SEQ ID NO:8, SEQ ID NO:10 et SEQ ID NO:12 et GPBP_{pep1} étant **caractérisé par** SEQ ID NO:14.
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- 55

GCAGGAAGATGCCGGCGGTAGCGGAGGTGTGAGTGGACGCGGGACTCAGCGGCCGGATTTCTCTTCCCT 70
TCTTTTCCCTTTTCCCTCCCTATTTGAAATTGGCATCGAGGGGGCTAAGTTCGGGTGGCAGCGCCGGGCG 140
CAACGCAGGGGTCACGGCGACGGCGGGCGGGCTGACGGCTGGAAGGGTAGGCTTCATTCCACCGCTCGTC 210
CTCCTTCCCTCGCTCCGCTCGGTGTCAGGCGCGGGCGGGCGGGCGGGCGGGACTTCGTCCCTCCTCTGC 280
TCCCCCCCACACCGGAGCGGGCACTCTTCGCTTCGCCATCCCCGACCCTTCACCCCGAGGACTGGGCGC 350
CTCCTCCGGCGCAGCTGAGGGAGCGGGGGCCGGTCTCCTGCTCGGTTGTCGAGCCTCCATGTCGGATAAT 420
M S D N 4
CAGAGCTGGAACTCGTCCGGCTCGGAGGAGGATCCAGAGACGGAGTCTGGGCCGCCTGTGGAGCGCTGCG 490
Q S W N S S G S E E D P E T E S G P P V E R C 27
GGGTCTCAGTAAGTGGACAACTACATTCATGGGTGGCAGGATCGTTGGGTAGTTTTGAAAAATAATGC 560
G V L S K W T N Y I H G W Q D R W V V L K N N A 51
TCTGAGTTACTACAAATCTGAAGATGAAACAGAGTATGGCTGCAGAGGATCCATCTGTCTTAGCAAGGCT 630
L S Y Y K S E D E T E Y G C R G S I C L S K A 74
GTCATCACACCTCACGATTTTGATGAATGTGCGATTTGATATTAGTGTAATGATAGTGTGGTATCTTC 700
V I T P H D F D E C R F D I S V N D S V W Y L 97
GTGCTCAGGATCCAGATCATAGACAGCAATGGATAGATGCCATTGAACAGCACAAAGACTGAATCTGGATA 770
R A Q D P D H R Q Q W I D A I E Q H K T E S G Y 121
TGGATCTGAATCCAGCTTGGCTCGACATGGCTCAATGGTGTCCCTGGTGTCTGGAGCAAGTGGCTACTCT 840
G S E S S L R R H G S M V S L V S G A S G Y S 144
GCAACATCCACCTCTTCATTCAAGAAAGGCCACAGTTTACGTGAGAAGTTGGCTGAAATGGAAACATTTA 910
A T S T S S F K K G H S L R E K L A E M E T F 167
GAGACATCTTATGTAGACAAGTTGACACGCTACAGAAGTACTTTGATGCCTGTGCTGATGCTGTCTCTAA 980
R D I L C R Q V D T L Q K Y F D A C A D A V S K 191
GGATGAACTTCAAAGGGATAAAGTGGTAGAAGATGATGAAGATGACTTTCTTACAACGCGTTCTGATGGT 1050
D E L Q R D K V V E D D E D D F P T T R S D G 214
GACTTCTTGCATAGTACCAACGGCAATAAAGAAAAGTTATTCCACATGTGACACCAAAGGAATTAATG 1120
D F L H S T N G N K E K L F P H V T P K G J N 237
GTATAGACTTTAAAGGGGAAGCGATAACTTTTAAAGCAACTACTGCTGGAATCCTTGCAACACTTTCTCA 1190
G I L F K G E A I T F K A T T A G I L A T L S H 261
TTGTATTGAACTAATGGTTAAACGTGAGGACAGCTGGCAGAAGAGACTGGATAAGGAAACTGAGAAGAAA 1260
C I E L M V K R E D S W Q K R L D K E T E K K 284
AGAAGAACAGAGGAAGCATATAAAAATGCAATGACAGAACTTAAGAAAAAATCCCACTTTGGAGGACCAG 1330
R R T E E A Y K N A M T E L K K K S H F G G P 307
ATTATGAAGAAGGCCCTAACAGTCTGATTAATGAAGAAGAGTTCTTTGATGCTGTTGAAGCTGCTCTTGA 1400
D Y E E G P N S L I N E E E F F D A V E A A L D 331

FIG. 1

EP 1 144 650 B1

CAGACAAGATAAAATAGAAGAACAGTCCACAGAGTGAAAAGGTGAGATTACATTGGCCTACATCCTTGCCC 1470
R Q D K I E E Q S Q S E K V R L H W P T S L P 354

TCTGGAGATGCCTTTTCTTCTGTGGGACACATAGATTTGTCCAAAAGCCCTATAGTCGCTCTTCCTCCA 1540
S G D A F S S V G T H R F V Q K P Y S R S S S 377

TGTCTTCCATTGATCTAGTCAGTGCCTCTGATGATGTTACAGATTGAGCTCCAGGTTGAAGAGATGGT 1610
M S S I D L V S A S D D V H R F S S Q V E E M V 401

GCAGAACCACATGACTTACTCATTACAGGATGTAGGCGGAGATGCCAATTGGCAGTTGGTTGTAGAAGAA 1680
Q N H M T Y S L Q D V G G D A N W Q L V V E E 424

GGAGAAATGAAGGTATACAGAAGAGAAGTAGAAGAAAATGGGATTGTTCTGGATCCTTTAAAAGCTACCC 1750
G E M K V Y R R E V E E N G I V L D P L K A T 447

ATGCAGTTAAAGGCGTCCACAGGACATGAAGTCTGCAATTATTTCTGGAATGTTGACGTTGCAATGACTG 1820
H A V K G V T G H E V C N Y F W N V D V R N D W 471

GGAAACAACTATAGAAAACCTTTCATGTGGTGGAAACATTAGCTGATAATGCAATCATCATTATCAAACA 1890
E T T I E N F H V V E T L A D N A I I I Y Q T 494

CACAAGAGGGTGTGGCCTGCTTCTCAGCGAGACGTATTATATCTTTCTGTCAATTCGAAAGATACCAGCCT 1960
H K R V W P A S Q R D V L Y L S V I R K I P A 517

TGACTGAAAATGACCCTGAAACTTGGATAGTTTGTAATTTTCTGTGGATCATGACAGTGCTCCTCTAAA 2030
L T E N D P E T W I V C N F S V D H D S A P L N 541

CAACCGATGTGTCCGTGCCAAAATAAATGTTGCTATGATTTGTCAAACCTTGGTAAGCCCACCAGAGGGA 2100
N R C V R A K I N V A M I C Q T L V S P P E G 564

AACCAGGAAATTAGCAGGGACAACATTCTATGCAAGATTACATATGTAGCTAATGTGAACCCTGGAGGAT 2170
N Q E I S R D N I L C K I T Y V A N V N P G G 587

GGGCACCAGCCTCAGTGTAAAGGGCAGTGGCAAAGCGAGAGTATCCTAAATTTCTAAAACGTTTTACTTC 2240
W A P A S V L R A V A K R E Y P K F L K R F T S 611

TTACGTCCAAGAAAAAAGTGCAGGAAAGCCTATTTTGTCTAGTATTAACAGGTAAGATATGTTT 2310
Y V Q E K T A G K P I L F 624

TATCTTTTTTTAACTTTTATTTGACTAATATGACTGTCAATACTAAAATTTAGTTGTTGAAAGTATTTACT 2380

ATGTTTTTTT 2389

FIG. 1

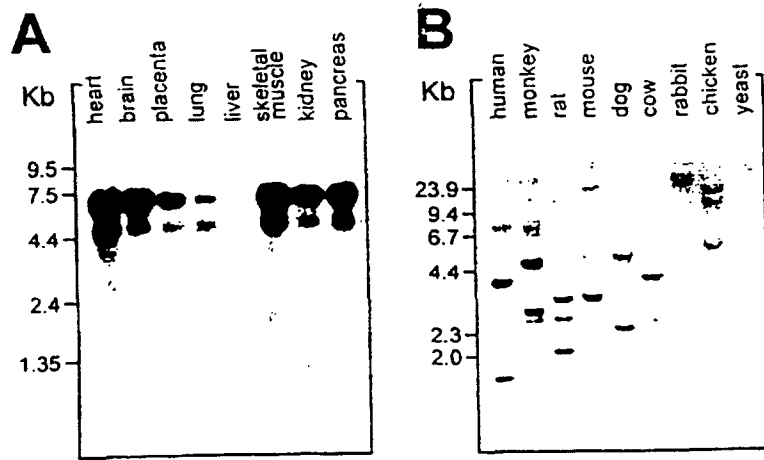


FIG. 2

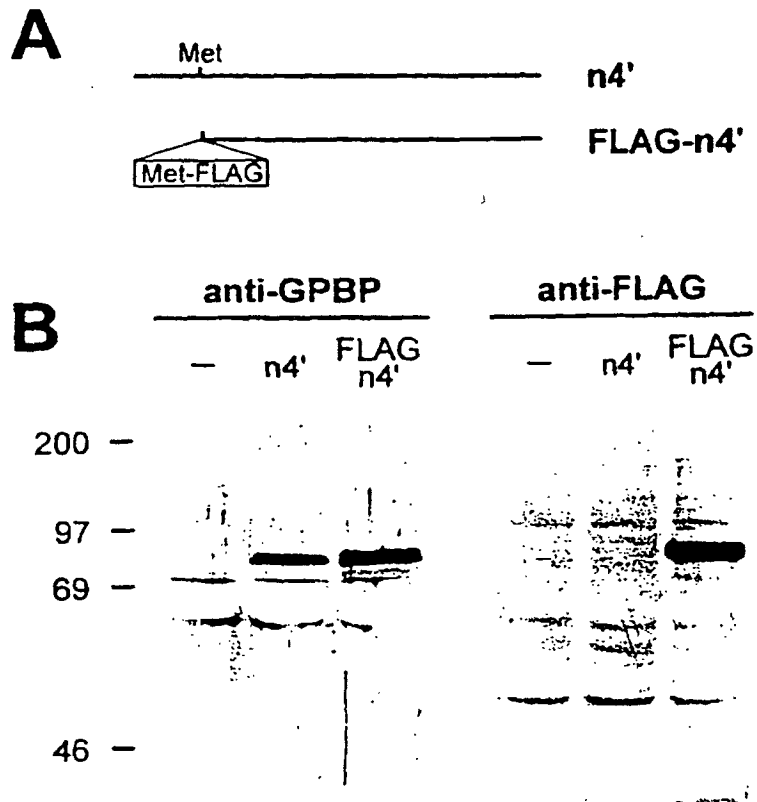


FIG. 3

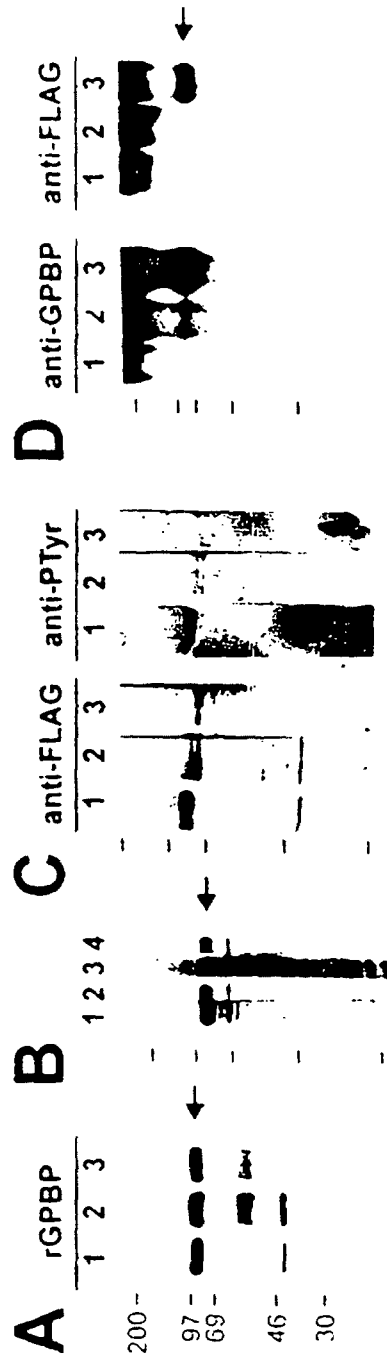


FIG. 4

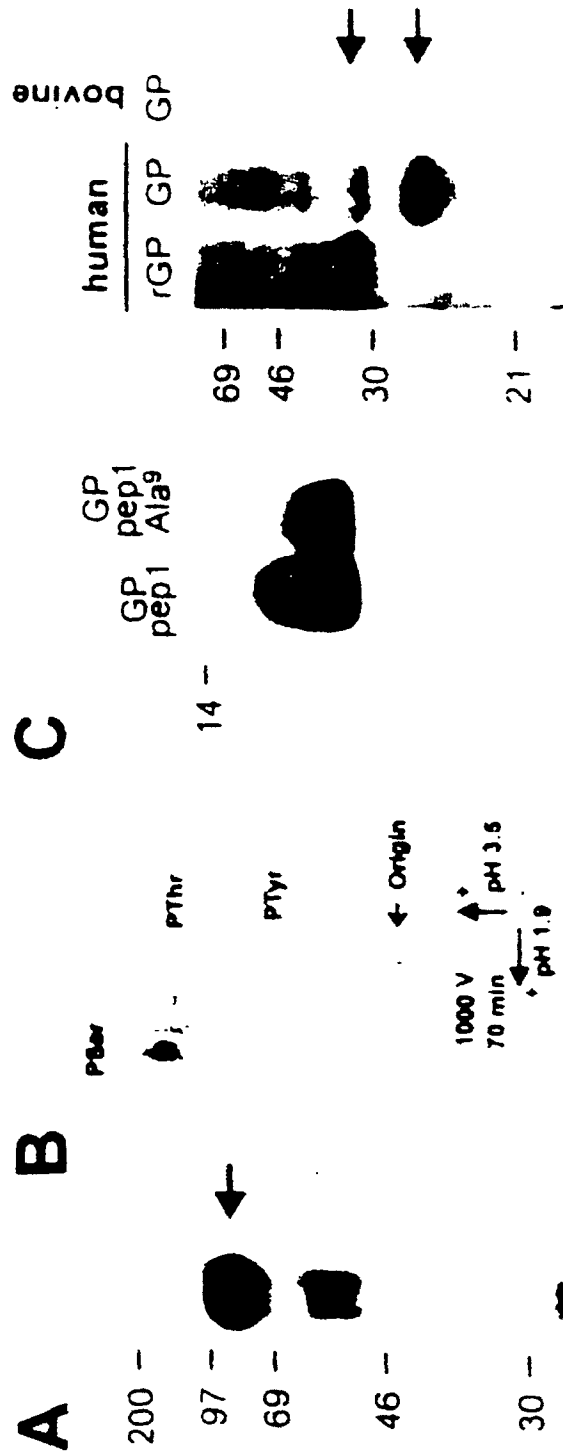


FIG. 5

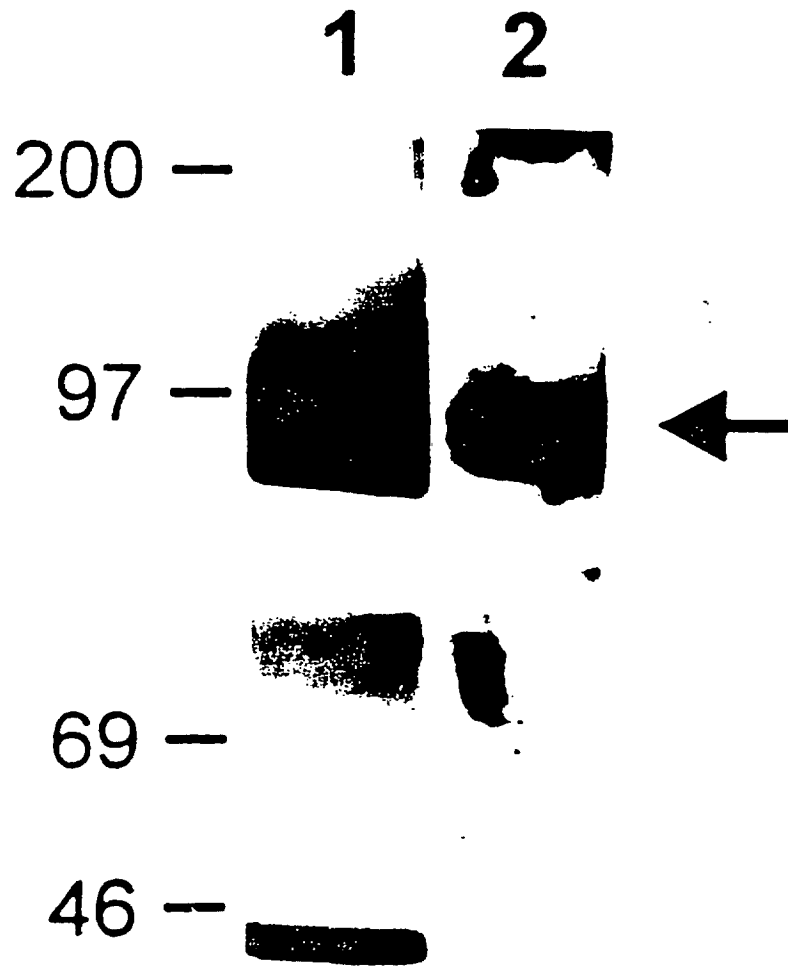


FIG. 6

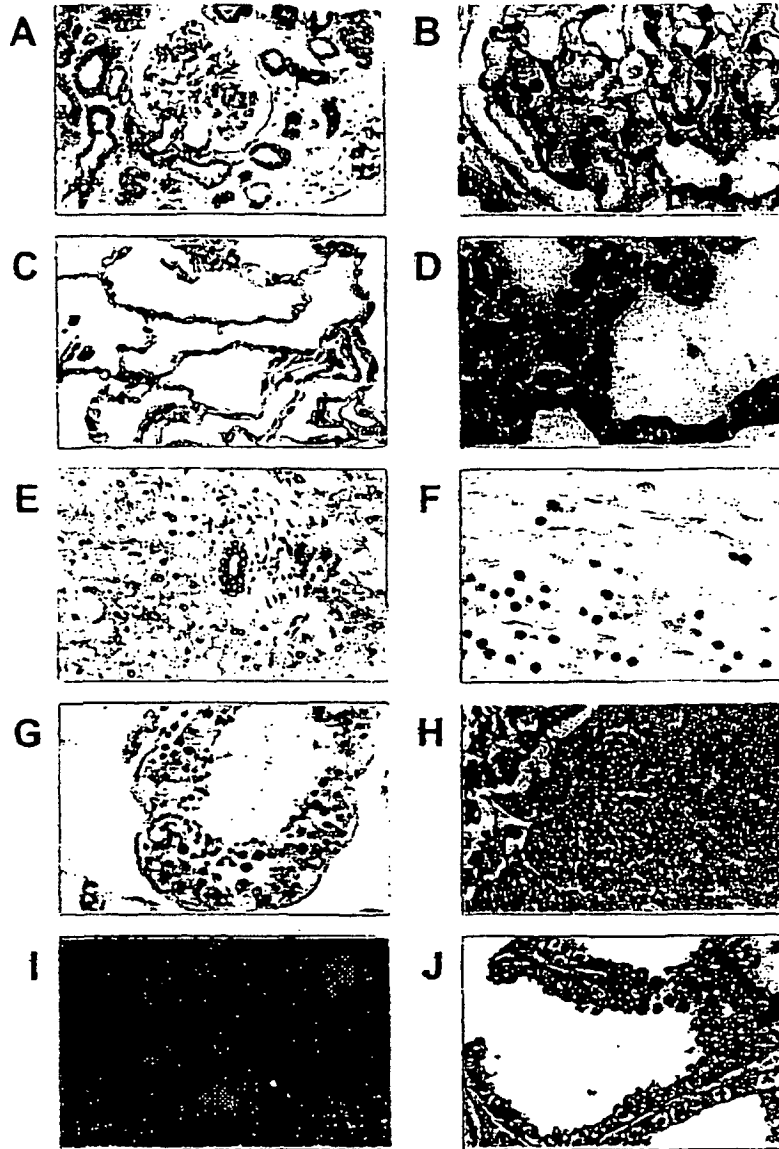


FIG. 7

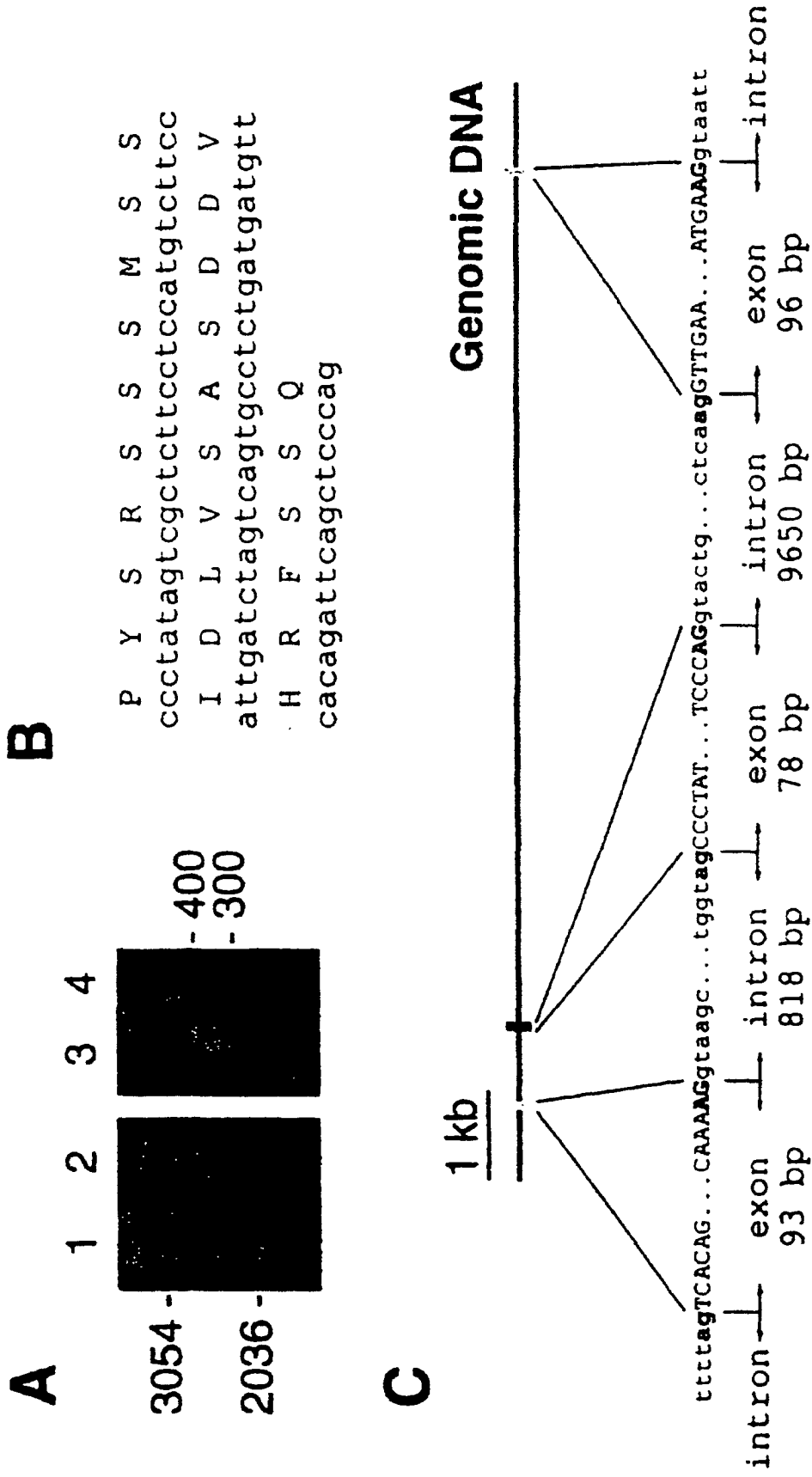


FIG. 8

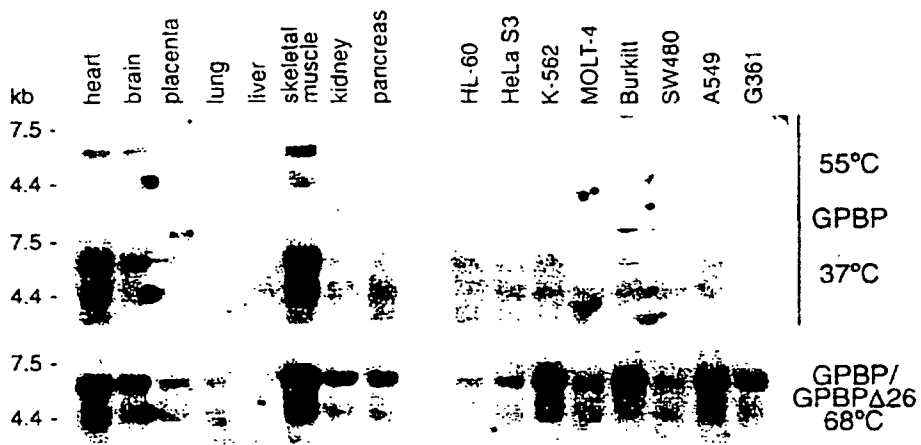


FIG. 9



FIG. 10

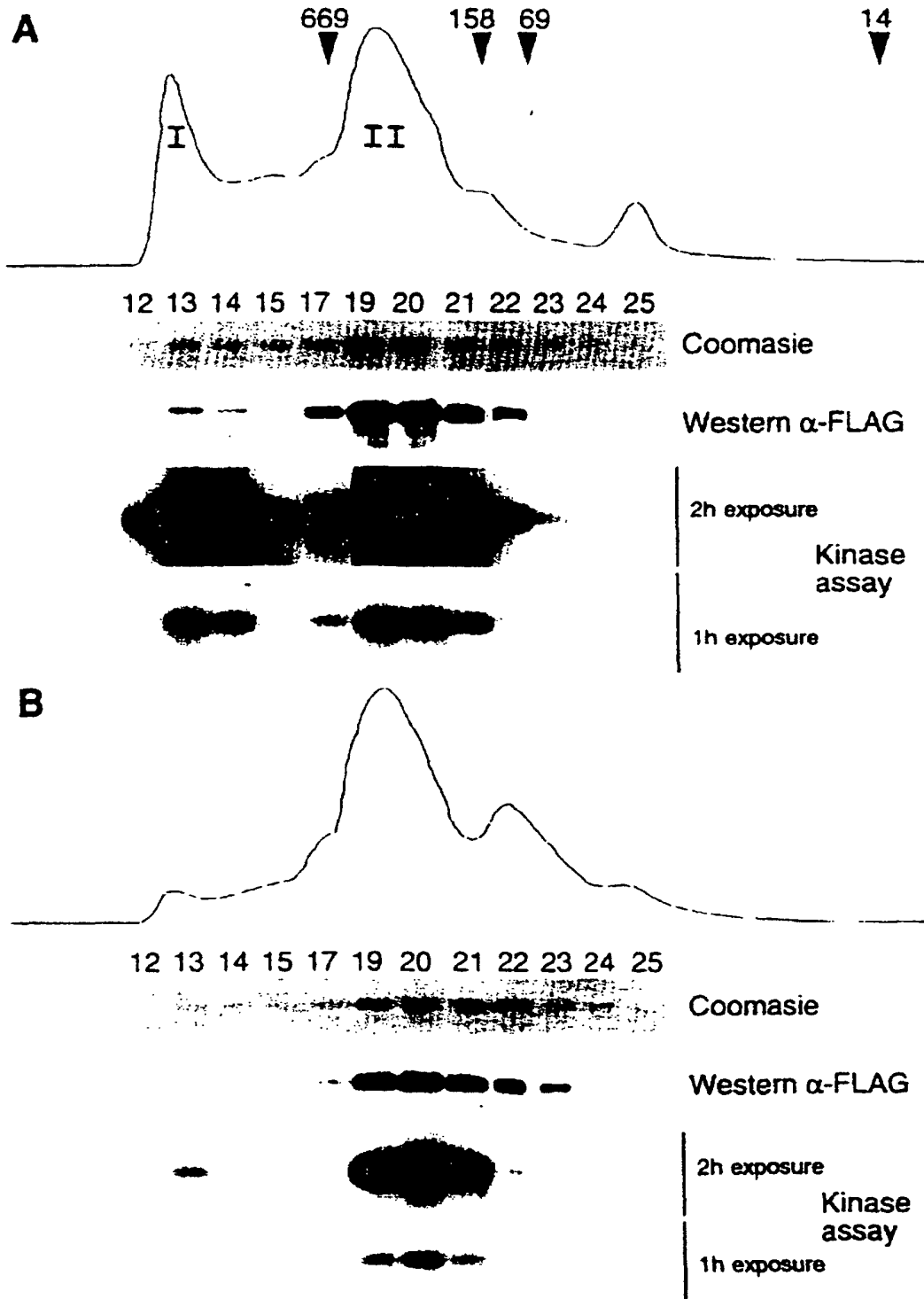


FIG. 11

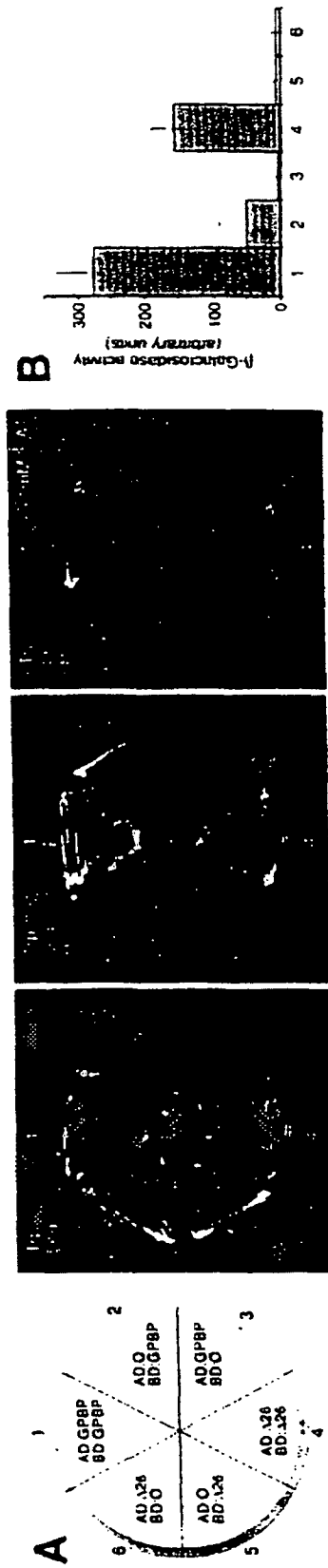


FIG. 12

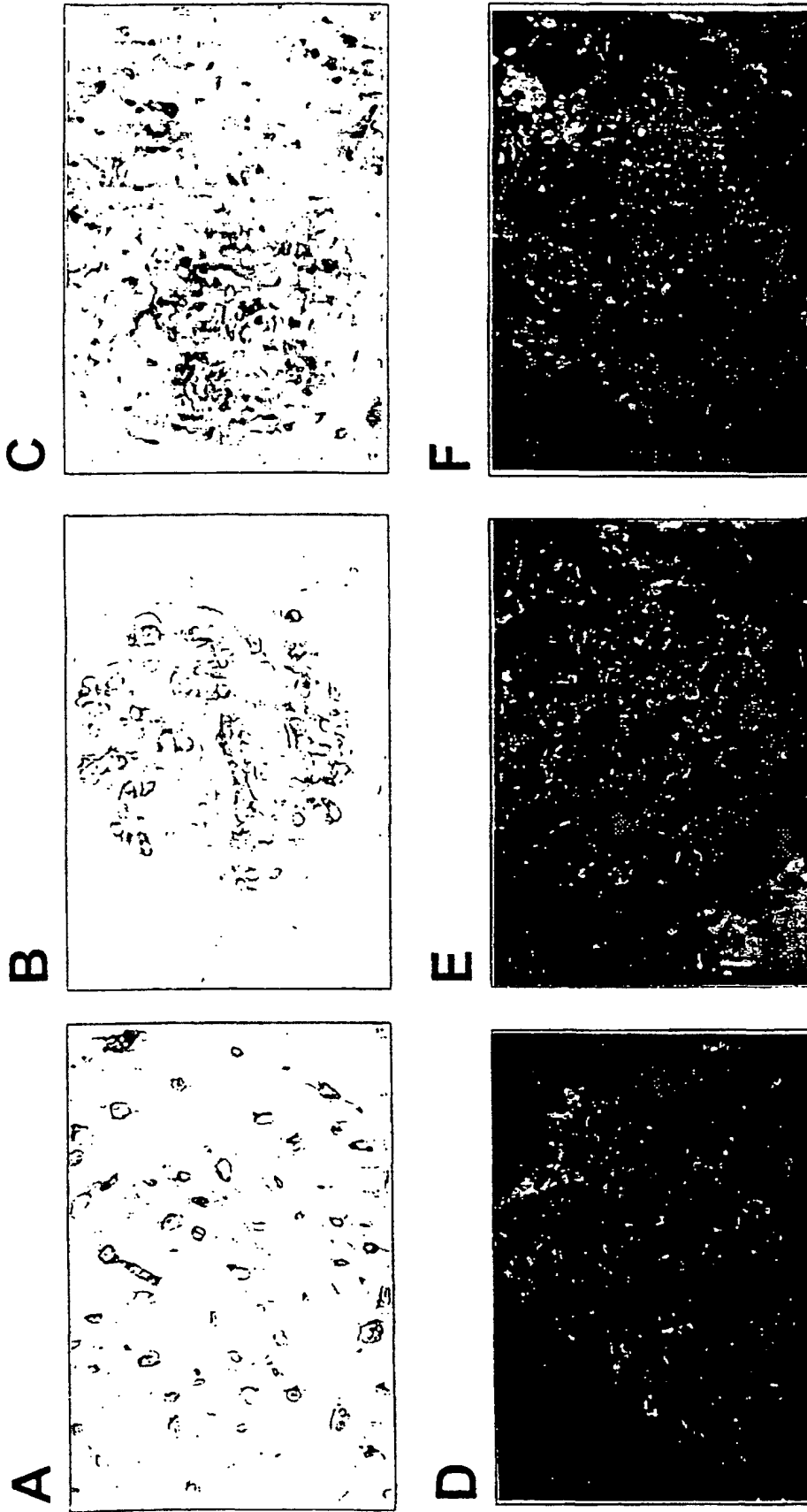


FIG. 13

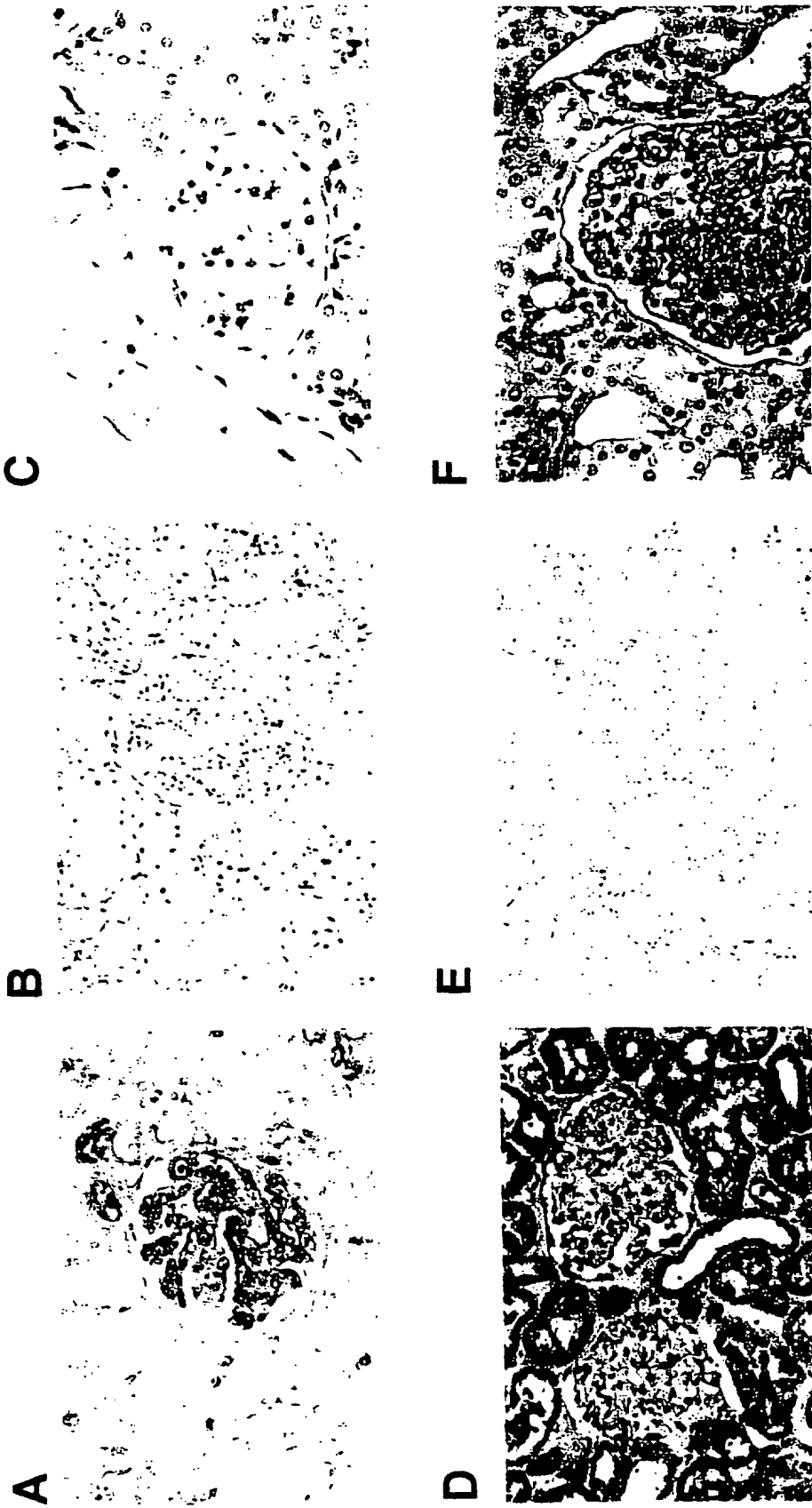


FIG. 14

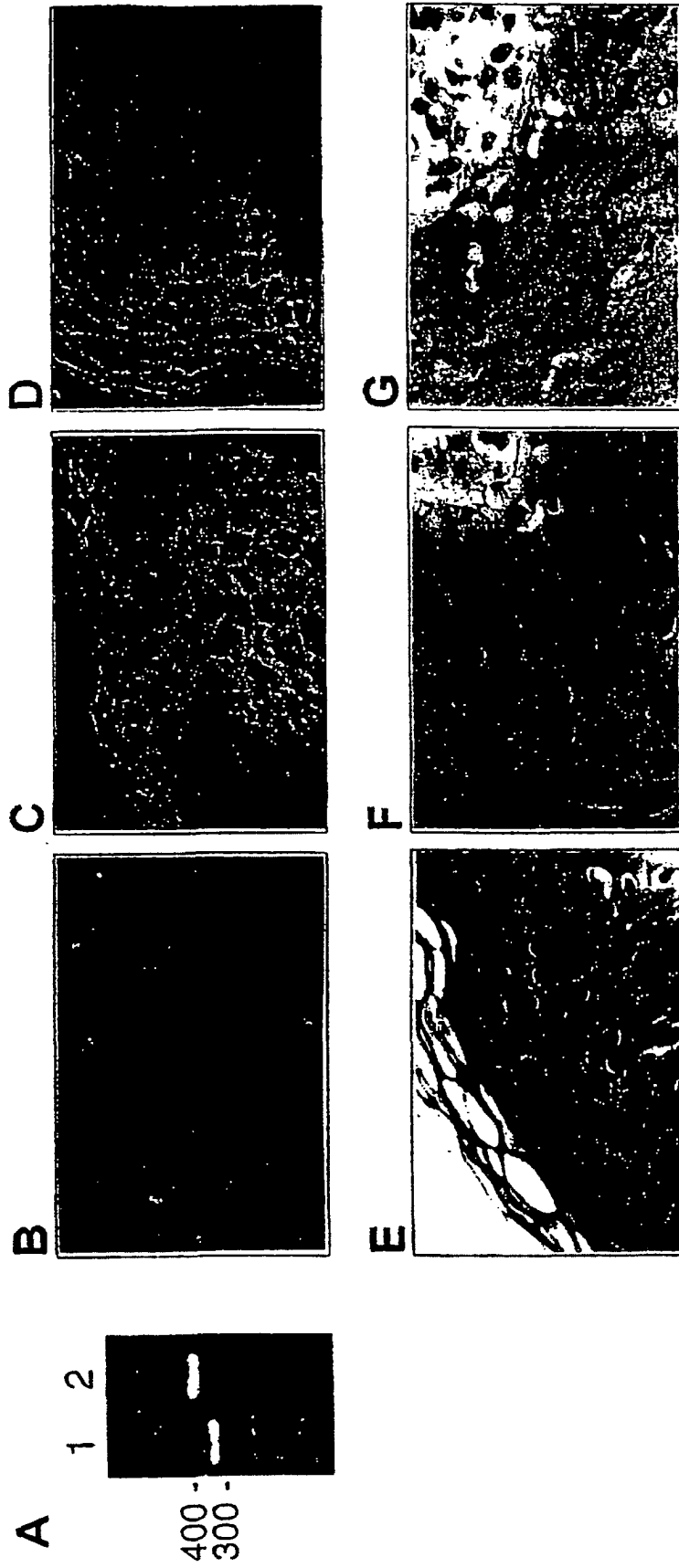


FIG. 15

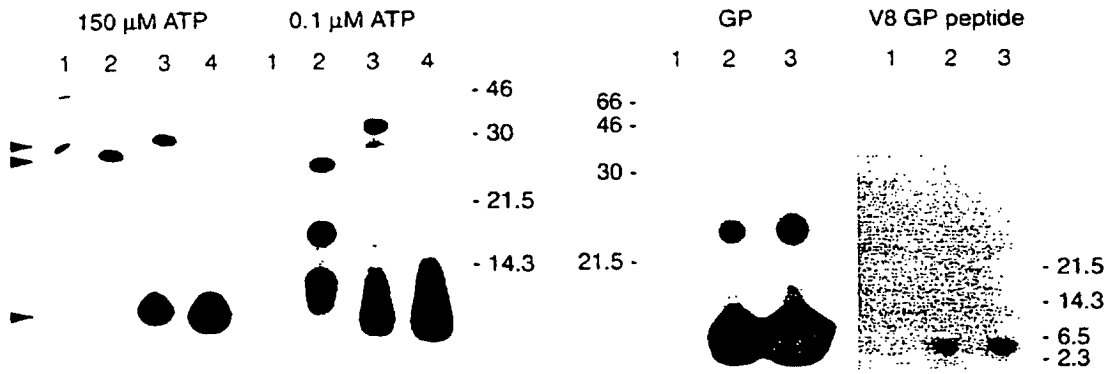


FIG. 16

| | |
|--------|--|
| GPΔIII | GLK <u>G</u> KRGDS <u>S</u> GS PATWTTRGFVFTRHSQTTAI |
| MBP | MAS <u>Q</u> KRP- <u>S</u> QRHGSKYLATASTMDHARHGFL |
| GPΔIII | PSCPEGPVPLYSGFSFLFVQGNQRAHGQDL <u>D</u> |
| MBP | PRHRDTGILDSIGRFFGGDRGAPKRGSGK-- |
| GPΔIII | A <u>L</u> FV <u>K</u> V <u>L</u> R <u>S</u> P |
| MBP | V <u>P</u> W <u>L</u> K <u>P</u> G <u>R</u> S <u>P</u> |

FIG. 17

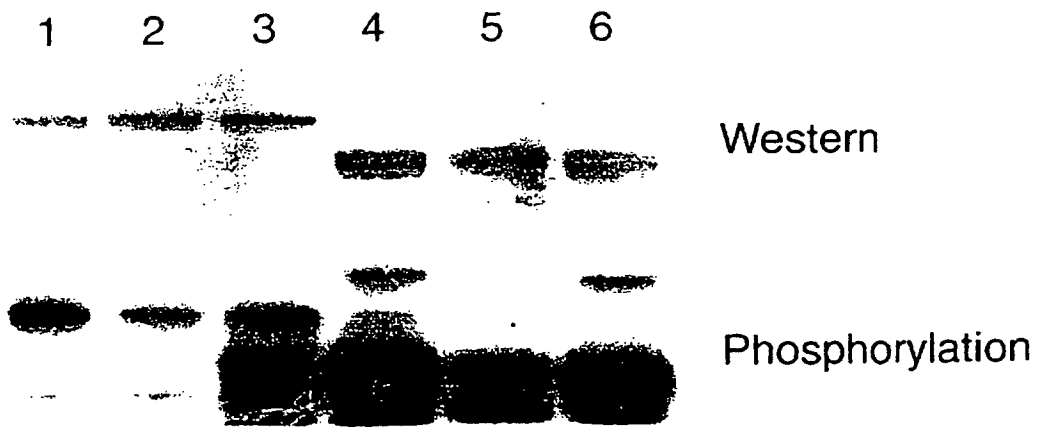


FIG. 18

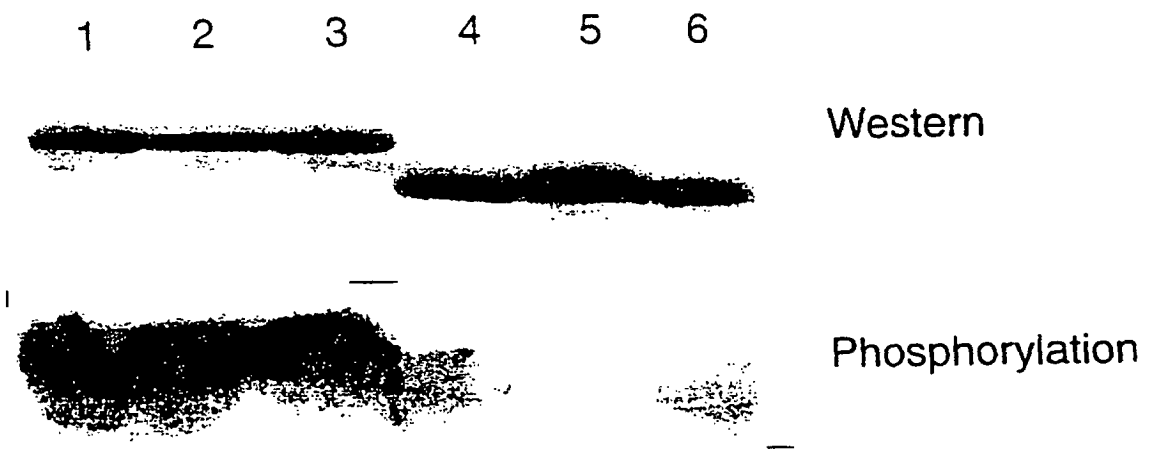


FIG. 19

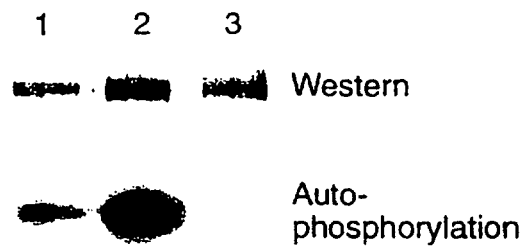


FIG. 20

| | | | |
|----------------|--|---------|------------|
| 专利名称(译) | Goodpasture抗原结合蛋白 | | |
| 公开(公告)号 | EP1144650B1 | 公开(公告)日 | 2006-01-25 |
| 申请号 | EP2000911146 | 申请日 | 2000-02-24 |
| [标]申请(专利权)人(译) | SAUS JUAN | | |
| 申请(专利权)人(译) | SAUS , JUAN | | |
| 当前申请(专利权)人(译) | SAUS , JUAN | | |
| [标]发明人 | SAUS JUAN | | |
| 发明人 | SAUS, JUAN | | |
| IPC分类号 | C12N15/54 C12N9/12 C07K16/40 C12Q1/48 C12Q1/68 A61K38/45 A61P35/00 A61P37/00 G01N33/50 A61K38/00 A61K39/00 A61P37/06 C07K14/47 C07K16/18 C12N1/15 C12N1/19 C12N1/21 C12N5/10 C12N15/09 C12P21/08 C12Q1/02 G01N33/15 G01N33/53 G01N33/564 | | |
| CPC分类号 | A61K38/00 A61P35/00 A61P37/00 A61P37/06 C07K16/40 C12N9/1205 C12Y207/01037 G01N33/564 | | |
| 代理机构(译) | Grund的 , MARTIN | | |
| 优先权 | 60/121483 1999-02-24 US | | |
| 其他公开文献 | EP1144650A2 | | |
| 外部链接 | Espacenet | | |

摘要(译)

本发明提供了分离的核酸序列和编码Goodpasture抗原结合蛋白 (GPBP) 的表达载体,基本上纯化的GPBP,针对GPBP的抗体,以及检测GPBP的方法。

TCGATTCACATGGCCCACTAGCCGATCAAGGACGAGATG

ACAAG (SEQ ID NO: 28)