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(54) RPDL protein and DNA encoding the same

RPDL Protein und kodierende DNS

Protéine "RPDL", et ADN codant ladite

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Description

Background of the Invention

5 Field of the Invention

[0001] The present invention relates to an RPDL protein which is a novel transcriptional control protein, a process for producing this protein, a method of using the same, a DNA encoding the protein, and a gene analysis method using the DNA. The present invention finds applications in the pharmaceutical field.

10

Description of the Related Art

[0002] Many genes execute selective expression, for example, at a specific time or site or when a certain stimulus has been given. The expression of the genes involves two important steps consisting of producing a mRNA on the 15 basis of information stored in the DNA sequence (transcription) and producing a protein by the action of the mRNA (translation).

[0003] It is becoming apparent in recent years that the transcription of genes in eukaryotic cells is skillfully controlled by a plurality of proteins known as transcriptional control proteins.

[0004] Analyzing in detail the mechanism of the above transcriptional control is a task extremely important from the 20 viewpoint of learning the selective expression control mechanism of genes, namely, the cell differentiation or amplification or various gene activities and ultimately the fundamental system relating to, for example, life and death. It is expected that the analysis of the mechanism of the above transcriptional control would break through difficult problems of not only tumors but also other various diseases and abnormalities, and further aging, dementia, obesity, etc.

[0005] For the elucidation of the transcriptional control mechanism, it is essential to achieve "understanding the 25 material bases of associated factors (transcriptional control protein, etc.)", "understanding the interaction between such factors", "understanding the whole process through a plurality of interactions", and "working out a systematic understanding through commonality and diversity" [see Masami Horikoshi et al, Tanpaku-shitsu • Kakusan • Koso (Protein, Nucleic Acid and Enzyme), Vol.38, No.5, p.p.831-841 (1993)].

[0006] Studies on structural fundamentals such that some basic transcription factors recognize specific sequences 30 of the DNA and bind therewith have been advanced with the use of viruses, bacteria, yeasts, and the like. However, for example, the number of constituent factor groups is so large that elucidation is still being awaited in various fields such as the interaction between factors, the interaction of the factor with a component of transcription initiation complex, such as RNA polymerase, and the commonality in the control mechanism between viruses, bacteria, yeasts and human. Therefore, a marked progress of the analysis described above based on the recent gene isolations, especially, the 35 cDNA clonings of factors associated with the human transcriptional control mechanism is being expected.

[0007] Known transcriptional control proteins include those specific for some genes and those commonly acting on a wide variety of genes. From the viewpoint of function, the known transcriptional control proteins include not only those capable of activating the transcription or inactivating the same but also those having both of the above capabilities 40 [see M. Ptashne, Scientific American, Vol.260, p.p.40-47 (1989)].

[0008] Up to now studies on eukaryotic cells in this field have been conducted with the use of yeast as the model 45 from the practical point of view, and it has been suggested that the fundamental mechanism thereof applies to human cells as well. The transcriptional control protein not only commonly acting on many genes but also having both the functions of activation and inactivation is considered as being especially important and, therefore, it is apparent that the studies on the effects exerted by its mutation with the use of yeast only have reached a limit.

[0009] Accordingly, isolating a human gene encoding the above important transcriptional control protein and identifying the protein has an extremely important significance in that a marked progress can be realized in the direct elucidation of the transcriptional control mechanism of the cells of multicellular organisms having such aspects as development, differentiation and tissue, especially, human per se.

50 Disclosure of the Invention

Summary of the Invention

[0010] An object of the present invention is to provide an important human transcriptional control protein not only 55 commonly acting on many target genes but also having both the functions of activation and inactivation, and a gene encoding the protein. Another object of the present invention is to provide a gene analysis method useful for elucidative studies on the mechanism of control of human gene transcription and on the effects on human cells caused by the mutation of the gene encoding the human transcriptional control protein with the use of the transcriptional control

protein and DNA encoding the same.

[0011] The yeast transcription factor RPD3 controls not only the transcription of high- and low-affinity potassium transporter gene TRK2 but also the transcription of many genes including genes PHO5, STE6 and TY2 as the target. Further, it is known that the yeast PRD3 protein has both the functions of activation and inactivation [see M. Vidal and R.F. Gaber, Mol. Cel. Biol. Vol.11, p.p.6317-6327 (1991)].

[0012] The present inventors have determined the 5'-terminal nucleotide sequence of each clone derived from a cDNA library prepared from human fetal lung and have found a clone exhibiting homology with the sequence of the RPD3 gene of a yeast. Further, they have determined the DNA sequence of this clone and have obtained a full-length cDNA encoding a novel protein. It has been confirmed that the amino acid sequence of the protein encoded by this cDNA exhibits a significant similarity to that of the yeast RPD3 and this protein is a novel human transcriptional control protein that has never been reported.

[0013] Moreover, the present inventors have confirmed that the gene encoding this protein is an important gene which has expressed in all the studied human tissues excluding the brain by a gene analysis according to the Northern blotting technique using the above cDNA as a probe.

[0014] Furthermore, the present inventors have confirmed that the gene encoding this protein is localized at 1p34.1 on the short arm of the chromosome 1, the region where a deletion is recognized in mammary and gastric carcinomas, by the chromosomal mapping according to the FISH (fluorescent in situ hybridization) technique using the above cDNA as a probe.

[0015] The present invention has enabled not only the production of a transformant having, introduced thereinto, the cDNA encoding the above human transcriptional control protein or a DNA obtained by artificially mutating the same by introducing the cDNA or the DNA into a host such as *E. coli*, yeast, an insect cell and a mammal cell, but also the production of the above protein or its variant with the use of the transformant and further the production of an antibody capable of binding with the above protein or its variant. Moreover, the present invention has enabled, on the level of human cells, not only the analyses of the interaction between the above protein and other factors capable of binding therewith, human genes controlled as the target and the activation and inactivation functions of the above protein as the transcriptional control factor, but also studies of the effects caused by the mutation of the DNA encoding the above protein.

[0016] Thus, the present invention provides an RPDL protein having an amino acid sequence, said amino acid sequence comprising the whole of the amino acid sequence specified in sequence ID NO 1. In the above explanation, "the variant of said RPDL protein" is one of RPDL proteins, has an amino acid sequence comprising the whole or a part of an amino acid sequence which is identical with the one specified in sequence ID NO 1 except that one or more amino acids are added thereto, deleted therefrom or inserted thereinto, or that one or more amino acids are substituted for one or more amino acids contained in sequence ID NO 1, and acts in the same manner as that of said RPDL protein having an amino acid sequence comprising the whole of the amino acid sequence specified in sequence ID NO 1.

[0017] Further, the present invention provides a DNA encoding said RPDL protein; a vector which contains a DNA encoding said RPDL protein; a transformant having, introduced thereinto, said vector; a process for producing said RPDL protein, which comprises culturing said transformant and recovering an expression product thereof; and a polyclonal antibody or a monoclonal antibody capable of combining with said RPDL protein.

[0018] Furthermore, the present invention provides a DNA probe having a DNA sequence, said DNA sequence comprising the whole of the DNA sequence specified in sequence ID NO 2 or comprising a sequence complementary to the whole of the DNA sequence specified in sequence ID No 2, and a gene analysis method for an RPDL protein characterized by hybridizing said DNA probe with a subject DNA.

[0019] In other words, the present invention relates to (1) a protein comprising the whole of the protein represented by sequence ID NO 1; (2) a DNA comprising the whole of the DNA represented by sequence ID NO 2, (3) a plasmid including the above DNA and a transformant carrying the same, (4) a process for producing the above protein, (5) an antibody capable of binding with the above protein, and (6) a gene analysis method or gene amplification method characterized by using the same.

[0020] The present invention will be described in detail below.

50 Detailed Description of the Invention

(1) Isolation of cDNA clone and confirmation of nucleotide sequence and amino acid sequence

[0021] cDNA was synthesized on the basis of mRNA derived from human fetal lung and a cDNA library containing cloned cDNA inserts in a given direction was prepared. The nucleotide sequence of each clone of this library was determined partially from the 5'-terminal side and one clone having a nucleotide sequence homologous with the RPD3 gene of a yeast was obtained. Further, the whole nucleotide sequence of this clone was determined with the result that the desired full-length cDNA sequence was obtained.

[0022] The cDNA obtained by the above procedure was confirmed as having a novel DNA sequence represented by sequence ID NO 2 and the amino acid sequence of a novel protein encoded thereby was deduced as shown in sequence ID NO 1. The present inventors designated the protein having the sequence specified in sequence ID NO 1 as a RPDL protein, this designation being employed throughout this description.

5 [0023] The DNA of the present invention and a DNA complementary to said DNA can find applications in gene and gene expression analyses by the use of a part thereof as a primer or probe. The term "a part of the DNA" as used herein means a sequence of continuous at least six nucleotides, preferably at least eight nucleotides, still more preferably at least ten nucleotides, and most preferably 10 to 12 nucleotides or 15 to 25 nucleotides corresponding to (i.e., contained in or complementary to) the nucleotide sequence of the DNA according to the present invention. The 10 primer or probe which is an oligonucleotide or polynucleotide may contain also at least one nucleotide(s) not corresponding to the nucleotide sequence of the DNA encoding the RPDL protein.

15 [0024] The protein of the present invention can find applications in antibody preparation and agents for study and diagnosis containing such antibodies by the use of the whole or a part thereof as an epitope. The term "epitope" means an antigenic determinant of a polypeptide. It is well known that the epitope is generally composed of at least 5 amino acid residues and that a polypeptide composed of 6 amino acid residues combines with an antibody [see WO of PCT Patent Applications No. 8403564, published on Sep. 13, 1984 (Assignee: COMMONWEALTH SERUM LABS AND GEYSEN, H.M.)]. The term "a part of the protein" as used herein refers to a polypeptide comprising at least about 3 to 5 consecutive amino acid residues, preferably at least about 8 to 10 consecutive amino acid residues, and still more preferably at least about 11 to 20 consecutive amino acid residues on the basis of the amino acid sequence of the 20 protein of the present invention. Needless to say, use can be made of even a polypeptide comprising at least about 20 amino acid residues. The polypeptide described above may contain also at least one amino acid residues not corresponding to the amino acid sequence of the RPDL protein.

25 [0025] In addition, RPDL proteins which are substantially equivalent to the RPDL protein having an amino acid sequence specified in sequence ID NO 1 and which are obtained by addition, deletion, insertion or substitution of one or more constituent amino acid residues of the above protein may be used. As in the protein above, DNAs which are substantially equivalent to the DNA encoding the RPDL protein having an amino acid sequence specified in sequence ID NO 1 and which are obtained by addition, deletion, insertion or substitution of one or more constituent nucleotides of the above DNA, i.e., equivalents, may be used.

30 (2) Recombinant expression vector and preparation of transformant and protein

[0026] A transformant can be obtained by inserting the DNA of the present invention into a suitable vector and transfected this vector into suitable host cells. Human RPDL protein can be produced in a large quantity by culturing the transformant in the customary manner and separating from the resultant culture. More particularly, a recombinant expression vector can be prepared by religating the above DNA or a fragment thereof to a vector suitable for the expression downstream of the promoter according to the customary procedure in which a restriction enzyme and DNA ligase are employed. Examples of suitable vectors include plasmids pBR322 and pUC18 derived from Escherichia coli, plasmid pUB110 derived from Bacillus subtilis, plasmid pRB15 derived from yeast, bacteriophage vectors λ gt10 and λ gt11, and vector SV40. The vectors are not particularly limited as long as they can be replicated or amplified in the host. The promoter and terminator are also not particularly limited as long as they suit the host employed in the expression of the DNA sequence. Appropriate members thereof can be used in combination in accordance with the host. The DNA to be employed is not limited to the one having a DNA sequence specified in sequence ID NO 2. Use may be made of a DNA having a DNA sequence resulting from intentional or unintentional substitution, deletion, insertion and/or addition conducted individually or in combination at a part of the DNA sequence of sequence ID NO 2. 40 Further, use may be made of one chemically synthesized.

45 [0027] The obtained recombinant expression vector is introduced into a host in accordance with any of the competent cell method [see J. Mol. Biol., Vol.53, p.154 (1970)], the protoplast method [see Proc. Natl. Acad. Sci. USA, Vol.75, p. 1929 (1978)], the calcium phosphate method [see Science, Vol.221, p.551 (1983)], the in vitro packaging method [see Proc. Natl. Acad. Sci. USA, Vol.72, p.581 (1975)], the virus vector method [see Cell, Vol.37, p.1053 (1984)], etc., thereby preparing a transformant. Any of Escherichia coli, Bacillus subtilis, yeast, insect cells, animal cells and the like is used as the host. The obtained transformant is cultured in a medium suitable for the host. The culturing is generally conducted at 20 to 45°C and at pH of 5 to 8, in which aeration and agitation are executed according to necessity. The separation of the RPDL protein from the resultant culture and its purification may be conducted by an appropriate combination of conventional separation and purification methods. Examples of these conventional methods include salting out, solvent precipitation, dialysis, gel filtration, electrophoresis, ion exchange chromatography, affinity chromatography, and reversed-phase high-performance liquid chromatography.

(3) Preparation of antibody

[0028] Antibodies can be prepared by the conventional method in which the whole or a part of the RPDL protein is used as an antigen. For example, a polyclonal antibody can be prepared by giving a plurality of subcutaneous, intra-muscular, intraperitoneal or intravenous inoculations of the antigen to an animal such as a mouse, a guinea-pig and a rabbit to thereby satisfactorily immunize the same, collecting the blood specimen from the animal, and performing serum separation. In this procedure, commercially available adjuvants can be used.

[0029] A monoclonal antibody can be prepared by, for example, conducting the fusion of splenocytes of a mouse immunized with the RPDL protein with commercially available mouse myeloma cells to thereby prepare a hybridoma and either culturing the hybridoma followed by separation of the antibody from the resultant supernatant or administering the hybridoma to a mouse followed by separation of the antibody from the mouse ascites.

[0030] The RPDL protein as an antigen does not necessarily have to possess the whole amino acid structure but use may be made of a peptide having a partial structure of the protein, a variant or derivative of the protein, or a fusion peptide resulting from the fusion with another peptide. The method for preparing these is not critical and it may be biological or chemosynthetic.

[0031] The obtained antibody enables the identification and quantity determination of RPDL protein in human biospecimens and can be used in, for example, various agents.

[0032] The immunoassay of RPDL protein may be conducted in accordance with the generally known procedure and can be executed by, for example, any of the fluorescent antibody technique, passive agglutination and enzyme antibody technique.

(4) Analyses of mutation and abnormality of gene

[0033] Any mutation of a gene encoding the RPDL protein can be analyzed by the use of a probe comprising a restriction enzyme fragment of the DNA provided by the present invention or by the use of, as a primer, an oligonucleotide obtained by appropriately selecting a suitably positioned nucleotide sequence from the DNA and synthesizing therewith.

[0034] Also, any abnormality such as insertion and deletion in the gene of a specimen can be detected by the above analysis.

[0035] The Escherichia coli L1-3977 carrying the plasmid containing the DNA encoding the above RPDL protein was deposited with National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry under the accession number FERM BP-4805 on September 21, 1994.

[0036] The use of a substance including the whole or a part of each of the human RPDL protein and the DNA encoding the protein according to the present invention has enabled analyses on the level of human cells not only of the functions of the above protein as a transcriptional control factor and the gene per se but also of the effects of any variation of the above protein. It is apparent that the protein of the present invention is an important transcriptional control protein commonly acting on many target genes and having both functions of activation and inactivation from the viewpoint of the homology of its amino acid sequence with that of the yeast RPD3. The contribution of the above investigations to the elucidation of the fundamental working of human cells, such as differentiation, amplification, activity and life and death thereof, is being anticipated. Moreover, the sequence structure of the above gene and its location on the chromosome have been defined, so that it can be anticipated that its relationships with not only tumors but also other various diseases and abnormalities of the gene are elucidated and its application is found in the pharmaceutical field.

Examples

[0037] The present invention will be concretely described in detail with reference to the following Examples which in no way limit the scope of the invention.

(Example 1) Preparation of human fetal lung cDNA library

[0038] cDNA was synthesized on the basis of mRNA derived from the human fetal lung (purchased from Clontech) and a cDNA library containing cloned cDNA inserts in a given direction was prepared by the use of UniZAPxR vector kit (purchased from Stratagene).

(Example 2) Selection of clone

[0039] The nucleotide sequence of each of 2058 clones of the cDNA library prepared in Example 1 was partially determined from the 5'-terminal side. The resultant nucleotide sequences were compared with the known nucleotide

sequences of a data base to find out one clone L1-3977 having homology with the yeast RPD3. A partial sequence (256 bp) of the clone L1-3977 exhibited a homology of 60.2% with the RPD3 gene (Accession No. S66438, 1645 bp) of yeast (*Saccharomyces cerevisiae*) in the range of 176 bp.

5 (Example 3) Sequencing of full-length cDNA and characteristics of structure

[0040] The DNA sequence of the clone L1-3977 obtained in Example 2 was determined by the Dideoxy method [see F. Sanger et al., Proc. Natl. Acad. Sci. USA, Vol.74, p.p.5463-5467 (1977)]. As a result, it was found that the clone L1-3977 contained a full-length cDNA having a novel sequence specified in sequence ID NO 2. The amino acid sequence of a novel protein (sequence ID NO 1, RPDL protein) composed of 482 amino acid residues was deduced from an open reading frame formed of 64th to 1509th nucleotides of the above DNA sequence.

[0041] This amino acid sequence exhibited a homology of 60.0% with the RPD3 protein (Accession No. S22284 & P32561, 433 amino acid residues) of yeast (*S. cerevisiae*) in the range of 422 amino acid residues.

[0042] The nucleotide sequence of sequence ID NO 2 (2111 bp) exhibited a homology of 62.1% with the RPD3 gene (Accession No. S66438, 1645 bp) of yeast (*S. cerevisiae*) in the range of 1168 bp. Further, it exhibited a homology as high as 80.9% with the RPD3 homologue gene (Accession No. X78454, 1040 bp) of *Xenopus laevis* in the range of 1034 bp. A homology as high as 94.8% was recognized in the range of 343 amino acid residues between the protein (343 amino acid residues) encoded by the RPD3 homologue gene (Accession No. X78454, 1040 bp) of *Xenopus laevis* and the RPDL protein of the present invention.

[0043] The above homology data demonstrate that the RPDL protein of the present invention is an important human transcriptional control protein having the same functions as those of the RPD3 protein of a yeast. In addition, the nucleotide sequence (2111 bp) of sequence ID NO 2 has exhibited a homology as high as 78.9% with the nucleotide sequence of the 3'-noncoding region of proto-oncogene c-*tk*1 (chicken tyrosine kinase proto-oncogene) in a range as wide as 1534 bp, so that the importance of the RPDL protein of the present invention in the transcriptional control mechanism has also been supported from the recent information on the association of the gene 3'-noncoding region with the control of transcription and translation.

(Example 4) Analysis of expression in various human tissues

[0044] Expression analysis by Northern blot system (purchased from Clontech) was conducted with respect to various human tissue mRNAs with the use of the cDNA obtained in Example 3 as a probe. The conditions recommended by the manufacturer were obeyed on hybridization and washing, and autoradiography was conducted at -80°C for 16 hours. Actin was used as a control. As a result, expression was recognized in the form of a mRNA band having a size of about 2.4 kbp in all the studied tissues (heart, kidney, liver, lung, pancreas, placenta, skeletal muscle, large intestine, peripheral leukocyte, ovary, prostate, small intestine, spleen, testis, and thymus gland) except the brain. While expression scarcely occurred in the brain, relatively strong expression occurred in the heart, pancreas and testis and relatively weak expression in the kidney.

(Example 5) Chromosome mapping of the gene

[0045] The cDNA obtained in Example 3 was used as a probe for investigating the location of the gene encoding the RPDL protein of the present invention on the chromosome. That is, the location on the chromosome with which the above probe hybridized was determined by the FISH method [see Inazawa et al., Genomics, Vol.10, p.p.1075-1078 (1991)]. As a result, the location was identified as being at 1p34.1 on the short arm of the chromosome 1. This location was the one at which deletion was recognized in mammary carcinoma [see A. Borg et al., Genes Chromosome Cancer, Vol.5, p.p.311-320 (1992)] and gastric carcinoma [see T. Sano et al., Cancer Res., Vol.51, p.p.2926-2931 (1991)].

(Example 6) Construction of recombinant RPDL protein expression vector

[0046] A partial sequence including the protein coding region was amplified by the PCR with the use of the cDNA obtained in Example 3 as a template. BamHI and EcoRI cleavage sites were added to the 5'-terminus of one primer and the 5'-terminus of the other primer, respectively. The obtained amplification product was digested with BamHI and EcoRI. The resultant fragment was inserted into expression vector pGEX-2T (purchased from Pharmacia) preliminarily digested with BamHI and EcoRI, thereby constructing expression plasmid pGST-RPDL. *E. coli* DH5 α was transformed with the plasmid pGST-RPDL and resulting transformants were selected based on the ampicillin resistance, thereby obtaining a transformant capable of expressing a fusion protein of glutathione-S-transferase and RPDL protein.

(Example 7) Expression of recombinant RPDL protein and its purification

[0047] The transformant obtained in Example 6 was cultured, and a recombinant RPDL fusion protein was extracted from the resultant culture and purified.

5 [0048] Specifically, the transformant was cultured by shaking the same in 100 ml of LB medium (1% peptone, 0.5% yeast extract and 1% NaCl) at 37°C overnight. The resultant liquid culture was diluted tenfold with LB medium preliminarily heated to 37°C and the resulting dilution was further cultured at 37°C for 30 to 90 minutes, thereby obtaining a culture of logarithmic growth phase. Isopropyl β -D-thiogalactopyranoside was added to 1 ℓ of the culture so that the final concentration thereof was 1 mM, followed by culturing for 3 to 4 hours. The culture was centrifuged to thereby separate bacterial cells. 10 ml of a column buffer (150 mM NaCl, 16 mM Na₂HPO₄, 4mM NaH₂PO₄, pH 7.3) was added to bacterial cells transformed with the expression vector pGST-RPDL, followed by sonication. A soluble fraction of a supernatant resulting from the cell disruption was applied to a glutathione-Sepharose 4B column (purchased from Pharmacia). The column was washed with the column buffer and then elution was conducted with an eluent containing 5 mM reduced glutathione. The eluted fraction was analyzed and fractionated by SDS polyacrylamide electrophoresis.

10 15 As a result, a fraction in which the desired GST fusion protein of about 80 kDa was detected as a main band was obtained from the transformant constructed with the plasmid pGST-RPDL.

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Sequence Listing

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sequence ID NO: 1

sequence length: 482

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sequence type: amino acid

molecule type: protein

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topology: linear

original source

organism: Homo sapiens

20

immediate source

library: human fetal lung cDNA library

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sequence description

Met Ala Gln Thr Gln Gly Thr Arg Arg Lys Val Cys Tyr Tyr Tyr Asp

1 5 10 15

30

Gly Asp Val Gly Asn Tyr Tyr Tyr Gly Gln Gly His Pro Met Lys Pro

20 25 30

35

His Arg Ile Arg Met Thr His Asn Leu Leu Leu Asn Tyr Gly Leu Tyr

35 40 45

40

Arg Lys Met Glu Ile Tyr Arg Pro His Lys Ala Asn Ala Glu Glu Met

50 55 60

45

Thr Lys Tyr His Ser Asp Asp Tyr Ile Lys Phe Leu Arg Ser Ile Arg

65 70 75 80

50

Pro Asp Asn Met Ser Glu Tyr Ser Lys Gln Met Gln Arg Phe Asn Val

85 90 95

55

Gly Glu Asp Cys Pro Val Phe Asp Gly Leu Phe Glu Phe Cys Gln Leu
 5 100 105 110
 Ser Thr Gly Gly Ser Val Ala Ser Ala Val Lys Leu Asn Lys Gln Gln
 10 115 120 125
 Thr Asp Ile Ala Val Asn Trp Ala Gly Gly Leu His His Ala Lys Lys
 15 130 135 140
 Ser Glu Ala Ser Gly Phe Cys Tyr Val Asn Asp Ile Val Leu Ala Ile
 145 150 155 160
 Leu Glu Leu Leu Lys Tyr His Gln Arg Val Leu Tyr Ile Asp Ile Asp
 20 165 170 175
 Ile His His Gly Asp Gly Val Glu Glu Ala Phe Tyr Thr Thr Asp Arg
 25 180 185 190
 Val Met Thr Val Ser Phe His Lys Tyr Gly Glu Tyr Phe Pro Gly Thr
 30 195 200 205
 Gly Asp Leu Arg Asp Ile Gly Ala Gly Lys Gly Lys Tyr Tyr Ala Val
 210 215 220
 35 Asn Tyr Pro Leu Arg Asp Gly Ile Asp Asp Glu Ser Tyr Glu Ala Ile
 225 230 235 240
 Phe Lys Pro Val Met Ser Lys Val Met Glu Met Phe Gln Pro Ser Ala
 40 245 250 255
 Val Val Leu Gln Cys Gly Ser Asp Ser Leu Ser Gly Asp Arg Leu Gly
 45 260 265 270
 Cys Phe Asn Leu Thr Ile Lys Gly His Ala Lys Cys Val Glu Phe Val
 50 275 280 285

	Lys Ser Phe Asn Leu Pro Met Leu Met Leu Gly Gly Gly Gly Tyr Thr			
5	290	295	300	
	Ile Arg Asn Val Ala Arg Cys Arg Thr Tyr Glu Thr Ala Val Ala Leu			
	305	310	315	320
10	Asp Thr Glu Ile Pro Asn Glu Leu Pro Tyr Asn Asp Tyr Phe Glu Tyr			
	325	330	335	
15	Phe Gly Pro Asp Phe Lys Leu His Ile Ser Pro Ser Asn Met Thr Asn			
	340	345	350	
20	Gln Asn Thr Asn Glu Tyr Leu Glu Lys Ile Lys Gln Arg Leu Phe Glu			
	355	360	365	
25	Asn Leu Arg Met Leu Pro His Ala Pro Gly Val Gln Met Gln Ala Ile			
	370	375	380	
	Pro Glu Asp Ala Ile Pro Glu Glu Ser Gly Asp Glu Asp Glu Asp Asp			
30	385	390	395	400
	Pro Asp Lys Arg Ile Ser Ile Cys Ser Ser Asp Lys Arg Ile Ala Cys			
	405	410	415	
35	Glu Glu Glu Phe Ser Asp Ser Glu Glu Glu Gly Glu Gly Arg Lys			
	420	425	430	
40	Asn Ser Ser Asn Phe Lys Lys Ala Lys Arg Val Lys Thr Glu Asp Glu			
	435	440	445	
	Lys Glu Lys Asp Pro Glu Glu Lys Lys Glu Val Thr Glu Glu Glu Lys			
45	450	455	460	
	Thr Lys Glu Glu Lys Pro Glu Ala Lys Gly Val Lys Glu Glu Val Lys			
50	465	470	475	480

Leu Ala

482

5 sequence ID NO: 2

10 sequence length: 2111

sequence type: nucleic acid

15 strandedness: double

topology: linear

molecule type: cDNA to mRNA,

20 original source

organism: Homo sapiens

25 immediate source

library: human fetal lung cDNA library

feature

30 feature key: CDS

location: 64..1512

35 identification of the feature: experimental examination

sequence description

40 GAGCGGAGCC GCAGGGCGGGA GGGCGGACGG ACCGACTGAC GGTAGGGACG GGAGGCGAGC 60

45 AAG ATG GCG CAG ACG CAG GGC ACC CGG AGG AAA GTC TGT TAC TAC TAC 108

Met Ala Gln Thr Gln Gly Thr Arg Arg Lys Val Cys Tyr Tyr Tyr

45 1 5 10 15

50 GAC GGG GAT GTT GGA AAT TAC TAT TAT GGA CAA GGC CAC CCA ATG AAG 156

Asp Gly Asp Val Gly Asn Tyr Tyr Gly Gln Gly His Pro Met Lys

55 20 25 30

5	CCT CAC CGA ATC CGC ATG ACT CAT AAT TTG CTG CTC AAC TAT GGT CTC	204
	Pro His Arg Ile Arg Met Thr His Asn Leu Leu Leu Asn Tyr Gly Leu	
	35 40 45	
10	TAC CGA AAA ATG GAA ATC TAT CGC CCT CAC AAA GCC AAT GCT GAG GAG	252
	Tyr Arg Lys Met Glu Ile Tyr Arg Pro His Lys Ala Asn Ala Glu Glu	
	50 55 60	
15	ATG ACC AAG TAC CAC AGC GAT GAC TAC ATT AAA TTC TTG CGC TCC ATC	300
	Met Thr Lys Tyr His Ser Asp Asp Tyr Ile Lys Phe Leu Arg Ser Ile	
	65 70 75	
20	CGT CCA GAT AAC ATG TCG GAG TAC AGC AAG CAG ATG CAG AGA TTC AAC	348
	Arg Pro Asp Asn Met Ser Glu Tyr Ser Lys Gln Met Gln Arg Phe Asn	
25	80 85 90 95	
	GTT GGT GAG GAC TGT CCA GTA TTC GAT GGC CTG TTT GAG TTC TGT CAG	396
	Val Gly Glu Asp Cys Pro Val Phe Asp Gly Leu Phe Glu Phe Cys Gln	
30	100 105 110	
	TTG TCT ACT GGT TCT GTG GCA AGT GCT GTG AAA CTT AAT AAG CAG	444
35	Leu Ser Thr Gly Gly Ser Val Ala Ser Ala Val Lys Leu Asn Lys Gln	
	115 120 125	
40	CAG ACG GAC ATC GCT GTG AAT TGG GCT GGG GGC CTG CAC CAT GCA AAG	492
	Gln Thr Asp Ile Ala Val Asn Trp Ala Gly Gly Leu His His Ala Lys	
	130 135 140	
45	AAG TCC GAG GCA TCT GGC TTC TGT TAC GTC AAT GAT ATC GTC TTG GCC	540
	Lys Ser Glu Ala Ser Gly Phe Cys Tyr Val Asn Asp Ile Val Leu Ala	
	145 150 155	
50		

5	ATC CTG GAA CTG CTA AAG TAT CAC CAG AGG GTG CTG TAC ATT GAC ATT	588
	Ile Leu Glu Leu Leu Lys Tyr His Gln Arg Val Leu Tyr Ile Asp Ile	
	160 165 170 175	
10	GAT ATT CAC CAT GGT GAC GGC GTG GAA GAG GCC TTC TAC ACC ACG GAC	636
	Asp Ile His His Gly Asp Gly Val Glu Glu Ala Phe Tyr Thr Thr Asp	
	180 185 190	
15	CGG GTC ATG ACT GTG TCC TTT CAT AAG TAT GGA GAG TAC TTC CCA GGA	684
	Arg Val Met Thr Val Ser Phe His Lys Tyr Gly Glu Tyr Phe Pro Gly	
	195 200 205	
20	ACT GGG GAC CTA CGG GAT ATC GGG GCT GGC AAA GGC AAG TAT TAT GCT	732
	Thr Gly Asp Leu Arg Asp Ile Gly Ala Gly Lys Gly Lys Tyr Tyr Ala	
25	210 215 220	
	GTT AAC TAC CCG CTC CGA GAC GGG ATT GAT GAC GAG TCC TAT GAG GCC	780
	Val Asn Tyr Pro Leu Arg Asp Gly Ile Asp Asp Glu Ser Tyr Glu Ala	
30	225 230 235	
	ATT TTC AAG CCG GTC ATG TCC AAA GTA ATG GAG ATG TTC CAG CCT AGT	828
	Ile Phe Lys Pro Val Met Ser Lys Val Met Glu Met Phe Gln Pro Ser	
35	240 245 250 255	
	GCG GTG GTC TTA CAG TGT GGC TCA GAC TCC CTA TCT GGG GAT CGG TTA	876
40	Ala Val Val Leu Gln Cys Gly Ser Asp Ser Leu Ser Gly Asp Arg Leu	
	260 265 270	
45	GGT TGC TTC AAT CTA ACT ATC AAA GGA CAC GCC AAG TGT GTG GAA TTT	924
	Gly Cys Phe Asn Leu Thr Ile Lys Gly His Ala Lys Cys Val Glu Phe	
	275 280 285	
50		

5	GTC AAG AGC TTT AAC CTG CCT ATG CTG ATG CTG GGA GGC GGT GGT TAC	972		
	Val Lys Ser Phe Asn Leu Pro Met Leu Met Leu Gly Gly Gly Tyr			
	290	295	300	
10	ACC ATT CGT AAC GTT GCC CGG TGC AGG ACA TAT GAG ACA GCT GTG GCC	1020		
	Thr Ile Arg Asn Val Ala Arg Cys Arg Thr Tyr Glu Thr Ala Val Ala			
	305	310	315	
15	CTG GAT ACG GAG ATC CCT AAT GAG CTT CCA TAC AAT GAC TAC TTT GAA	1068		
	Leu Asp Thr Glu Ile Pro Asn Glu Leu Pro Tyr Asn Asp Tyr Phe Glu			
20	320	325	330	335
	TAC TTT GGA CCA GAT TTC AAG CTC CAC ATC AGT CCT TCC AAT ATG ACT	1116		
	Tyr Phe Gly Pro Asp Phe Lys Leu His Ile Ser Pro Ser Asn Met Thr			
25	340	345	350	
	AAC CAG AAC ACG AAT GAG TAC CTG GAG AAG ATC AAA CAG CGA CTG TTT	1164		
30	Asn Gln Asn Thr Asn Glu Tyr Leu Glu Lys Ile Lys Gln Arg Leu Phe			
	355	360	365	
	GAG AAC CTT AGA ATG CTG CCG CAC GCA CCT GGG GTC CAA ATG CAG GCG	1212		
35	Glu Asn Leu Arg Met Leu Pro His Ala Pro Gly Val Gln Met Gln Ala			
	370	375	380	
40	ATT CCT GAG GAC GCC ATC CCT GAG GAG AGT GGC GAT GAG GAC GAA GAC	1260		
	Ile Pro Glu Asp Ala Ile Pro Glu Glu Ser Gly Asp Glu Asp Glu Asp			
	385	390	395	
45	GAC CCT GAC AAG CGC ATC TCG ATC TGC TCC TCT GAC AAA CGA ATT GCC	1308		
	Asp Pro Asp Lys Arg Ile Ser Ile Cys Ser Ser Asp Lys Arg Ile Ala			
50	400	405	410	415

5	TGT GAG GAA GAG TTC TCC GAT TCT GAA GAG GAG GGA GAG GGG GGC CGC	1356	
	Cys Glu Glu Glu Phe Ser Asp Ser Glu Glu Glu Gly Glu Gly Gly Arg		
	420	425	430
10	AAG AAC TCT TCC AAC TTC AAA AAA GCC AAG AGA GTC AAA ACA GAG GAT	1404	
	Lys Asn Ser Ser Asn Phe Lys Lys Ala Lys Arg Val Lys Thr Glu Asp		
	435	440	445
15	GAA AAA GAG AAA GAC CCA GAG GAG AAG AAA GAA GTC ACC GAA GAG GAG	1452	
	Glu Lys Glu Lys Asp Pro Glu Glu Lys Lys Glu Val Thr Glu Glu Glu		
	450	455	460
20	AAA ACC AAG GAG GAG AAG CCA GAA GCC AAA GGG GTC AAG GAG GAG GTC	1500	
	Lys Thr Lys Glu Glu Lys Pro Glu Ala Lys Gly Val Lys Glu Glu Val		
	465	470	475
25	AAG TTG GCC TGAATGGACC TCTCCAGCTC TGGCTTCCTG CTGAGTCCCT	1549	
	Lys Leu Ala		
30	480 482		
	CACGTTCTT CCCCCACCCC TCAGATTTA TATTTCTAT TTCTCTGTGT ATTTATATAA	1609	
35	AAATTTATTA AATATAAATA TCCCCAGGGA CAGAAACCAA GGCCCCGAGC TCAGGGCAGC	1669	
	TGTGCTGGGT GAGCTCTTCC AGGAGCCACC TTGCCACCCA TTCTTCCCGT TCTTAACCTT	1729	
	GAACCATAAA GGGTGCCAGG TCTGGGTGAA AGGGATACTT TTATGCAACC ATAAGACAAA	1789	
40	CTCCTGAAAT GCCAAGTGCC TGCTTAGTAG CTTTGGAAAG GTGCCCTTAT TGAACATTCT	1849	
	AGAAGGGGTG GCTGGGTCTT CAAGGATCTC CTGTTTTTT CAGGCTCCTA AAGTAACATC	1909	
45	AGCCATTTT AGATTGGTTC TGTTTCGTA CCTTCCCACT GGCCCTCAAGT GAGCCAAGAA	1969	
	ACACTGCCTG CCCTCTGTCT GTCTTCTCCT AATTCTGCAG GTGGAGGTTG CTAGTCTAGT	2029	
	TTCCTTTTG AGATACTATT TTCATTTTG TGAGCCTCTT TGTAATAAAA TGGTACATTT	2089	
50	CTAAAAAAAAA AAAAAAAAAA AA	2111	

55 **Claims**

1. An RPDL protein having an amino acid sequence, said amino acid sequence comprising the whole of the amino acid sequence specified in sequence ID NO 1.

2. The DNA encoding an RPDL protein as claimed in claim 1, which has the DNA sequence specified in sequence ID NO 2.

5 3. A vector which contains a DNA encoding an RPDL protein having an amino acid sequence, said amino acid sequence comprising the whole of the amino acid sequence specified in sequence ID NO 1.

4. A transformant having, introduced thereinto, a vector, said vector containing a DNA encoding an RPDL protein, said RPDL protein having an amino acid sequence comprising the whole of the amino acid sequence specified in sequence ID NO 1.

10 5. A process for producing an RPDL protein having an amino acid sequence comprising the whole of the amino acid sequence specified in sequence ID NO 1, which comprises culturing a transformant having, introduced thereinto, a vector containing a DNA encoding the RPDL protein, and recovering an expression product thereof.

15 6. A polyclonal antibody or a monoclonal antibody capable of combining with an RPDL protein having an amino acid sequence, said amino acid sequence comprising the whole of the amino acid sequence specified in sequence ID NO 1.

20 7. A gene analysis method for an RPDL protein **characterized by** hybridizing a DNA probe with a subject DNA, wherein said DNA probe has a DNA sequence comprising the whole of the DNA sequence specified in sequence ID NO 2.

25 8. A gene analysis method for an RPDL protein **characterized by** hybridizing a DNA probe with a subject DNA, wherein said DNA probe has a DNA sequence comprising a sequence complementary to the whole of the DNA sequence specified in sequence ID NO 2.

Patentansprüche

30 1. RPDL-Protein mit einer Aminosäuresequenz, wobei die Aminosäuresequenz die gesamte Aminosäuresequenz, dargestellt in SEQ ID NO: 1, umfasst.

2. DNA, codierend für ein RPDL-Protein gemäss Anspruch 1, das die in SEQ ID NO: 2 angegebene DNA-Sequenz aufweist.

35 3. Vektor, der eine DNA enthält, die für ein RPDL-Protein mit einer Aminosäuresequenz codiert, wobei die Aminosäuresequenz die gesamte Aminosäuresequenz, wie in SEQ ID NO: 1 dargestellt, umfasst.

4. Transformant, der darin eingeführt einen Vektor aufweist, wobei der Vektor eine DNA enthält, die für ein RPDL-Protein mit einer Aminosäuresequenz codiert, wobei die Aminosäuresequenz die gesamte Aminosäuresequenz, wie in SEQ ID NO: 1 dargestellt, umfasst.

40 5. Verfahren zur Erzeugung eines RPDL-Proteins mit einer Aminosäuresequenz, umfassend die ganze Aminosäuresequenz, wie in SEQ ID NO: 1 dargestellt, das das Kultivieren eines Transformanten umfasst, der einen Vektor enthält, enthaltend eine DNA, die das RPDL-Protein codiert, und Gewinnung eines Expressionsprodukts davon.

6. Polyklonaler Antikörper oder monoklonaler Antikörper, der zur Kombination mit einem RPDL-Protein mit einer Aminosäuresequenz fähig ist, wobei die Aminosäuresequenz die ganze Aminosäuresequenz umfasst, die in SEQ ID NO: 1 dargestellt ist.

45 7. Genanalyseverfahren für ein RPDL-Protein, **gekennzeichnet durch** Hybridisieren einer DNA-Sonde mit einer Ziel-DNA, wobei die DNA-Sonde eine DNA-Sequenz aufweist, umfassend die gesamte DNA-Sequenz, wie in SEQ ID NO: 2 dargestellt.

8. Genanalyseverfahren für ein RPDL-Protein, **gekennzeichnet durch** Hybridisieren einer DNA-Sonde mit einer Ziel-DNA, wobei die DNA-Sonde eine DNA-Sequenz aufweist, umfassend eine Sequenz, komplementär zu der ganzen DNA-Sequenz, wie in SEQ ID NO: 2 dargestellt.

Revendications

1. Protéine RPDL comportant une séquence d'acides aminés, ladite séquence d'acides aminés comprenant la totalité de la séquence d'acides aminés spécifiée dans la séquence à numéro d'identification 1.
5
2. ADN codant une protéine RPDL telle que revendiquée dans la revendication 1, qui comporte la séquence d'ADN spécifiée dans la séquence à numéro d'identification 2.
3. Vecteur qui contient un ADN codant une protéine RPDL comprenant une séquence d'acides aminés, ladite séquence d'acides aminés comprenant la totalité de la séquence d'acides aminés spécifiée dans la séquence à numéro d'identification 1.
10
4. Transformant comportant, introduit dans celui-ci, un vecteur, ledit vecteur contenant un ADN codant une protéine RPDL, ladite protéine RPDL comportant une séquence d'acides aminés comprenant la totalité de la séquence d'acides aminés spécifiée dans la séquence à numéro d'identification 1.
15
5. Procédé destiné à produire une protéine RPDL comportant une séquence d'acides aminés comprenant la totalité de la séquence d'acides aminés spécifiée dans la séquence à numéro d'identification 1, qui comprend la mise en culture d'un transformant comportant, introduit dans celui-ci, un vecteur contenant un ADN codant la protéine RPDL, et la récupération d'un produit d'expression de celle-ci.
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6. Anticorps polyclonal ou anticorps monoclonal capable de se combiner avec une protéine RPDL comportant une séquence d'acides aminés, ladite séquence d'acides aminés comprenant la totalité de la séquence d'acides aminés spécifiée dans la séquence à numéro d'identification 1.
25
7. Procédé d'analyse de gène destiné à une protéine RPDL **caractérisé par** l'hybridation d'une sonde d'ADN avec un ADN concerné, dans lequel ladite sonde d'ADN présente une séquence d'ADN comprenant la totalité de la séquence d'ADN spécifiée dans la séquence à numéro d'identification 2.
8. Procédé d'analyse de gène destiné à une protéine RPDL **caractérisé par** l'hybridation d'une sonde d'ADN avec un ADN concerné, dans lequel ladite sonde d'ADN comporte une séquence d'ADN comprenant une séquence complémentaire de la totalité de la séquence d'ADN spécifiée dans la séquence à numéro d'identification 2.
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