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(54) Titre : GENES DE POLY(ADP-RIBOSE)POLYMERASE
(54) Title: POLY(ADP-RIBOSE) POLYMERASE GENE

(57) Abrégé/Abstract:

The invention relates to poly(ADP-ribose)polymerase (PARP) homologues which are characterised by an amino acid sequence with a) a functional NAD⁺-binding site and b) no zinc-finger-sequence motif of general formula CX₂CX_mHX₂C, wherein m is an integral number 28 or 30 and the radicals X represent any amino acid, independently of each other; and to the functional equivalents of said poly(ADP-ribose)polymerase (PARP) homologues. The invention also relates to nucleic acids coding the poly(ADP-ribose)polymerase (PARP) homologues, to antibodies with specificity for the novel protein, to pharmaceutical and gene therapy agents containing the inventive products, to methods for analytically determining the inventive proteins and nucleic acids, to methods for identifying the effectors or bonding partners of the inventive proteins, to novel PARP effectors and to methods for determining the effectiveness of effectors of this type.

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(54) Title: POLY(ADP-RIBOSE)POLYMERASE GENE (54) Bezeichnung: POLY(ADP-RIBOSE)POLYMERASE-GENE (57) Abstract The invention relates to poly(ADP-ribose)polymerase (PARP) homologues which are characterised by an amino acid sequence with a) a functional NAD⁺-binding site and b) no zinc-finger-sequence motif of general formula CX₂CX_mHX₂C, wherein m is an integral number 28 or 30 and the radicals X represent any amino acid, independently of each other, and to the functional equivalents of said poly(ADP-ribose)polymerase (PARP) homologues. The invention also relates to nucleic acids coding the poly(ADP-ribose)polymerase (PARP) homologues, to antibodies with specificity for the novel protein, to pharmaceutical and gene therapy agents containing the inventive products, to methods for analytically determining the inventive proteins and nucleic acids, to methods for identifying the effectors or bonding partners of the inventive proteins, to novel PARP effectors and to methods for determining the effectiveness of effectors of this type. (57) Zusammenfassung Die Erfindung betrifft Poly(ADP-ribose)polymerase (PARP)-Homologe, gekennzeichnet durch eine Aminosäuresequenz, welche a) eine funktionale NAD⁺-Bindungsdomäne und b) kein Zink-Finger-Sequenzmotiv der allgemeinen Formel: CX₂CX_mHX₂C, aufweist, worin m für einen ganzzahligen Wert von 28 oder 30 steht und die Reste X unabhängig voneinander für eine beliebige Aminosäure stehen; und die funktionalen Äquivalente davon; dafür kodierende Nukleinsäuren; Antikörper mit Spezifität für die neuen Proteine; pharmazeutische und gentherapeutische Mittel, welche erfindungsgemäße Produkte enthalten; Verfahren zur analytischen Bestimmung der erfindungsgemäßen Proteine und Nukleinsäuren; Verfahren zur Identifizierung von Effektoren oder Bindungspartnern der erfindungsgemäßen Proteine; neuartige PARP-Effektoren; und Verfahren zur Bestimmung der Wirksamkeit solcher Effektoren.		

POLY(ADP-RIBOSE) POLYMERASE GENE

The present invention relates to novel poly(ADP-ribose) polymerase (PARP) genes and to the proteins derived therefrom; antibodies with specificity for the novel proteins; pharmaceutical and gene therapy compositions which comprise products according to the invention; methods for the analytical determination of the proteins and nucleic acids according to the invention; methods for identifying effectors or binding partners of the proteins according to the invention; methods for determining the activity of such effectors and use thereof for the diagnosis or therapy of pathological states.

In 1966, Chambon and co-workers discovered a 116 kD enzyme which was characterized in detail in subsequent years and is now called PARP (EC 2.4.2.30) (poly(adenosine-5'-diphosphoribose) polymerase), PARS (poly(adenosine-5'-diphosphoribose) synthase) or ADPRT (adenosine-5'-diphosphoribose transferase). In the plant kingdom (*Arabidopsis thaliana*) a 72kD (637 amino acids) PARP was found in 1995 (Lepiniec L. et al., FEBS Lett 1995; 364(2): 103-8). It was not clear whether this shorter form of PARP is a plant-specific individuality or an artefact ("splice" variant or the like). The 116 kD PARP enzyme has to date been unique in animals and in man in its activity, which is described below. It is referred to as PARP1 below to avoid ambiguity.

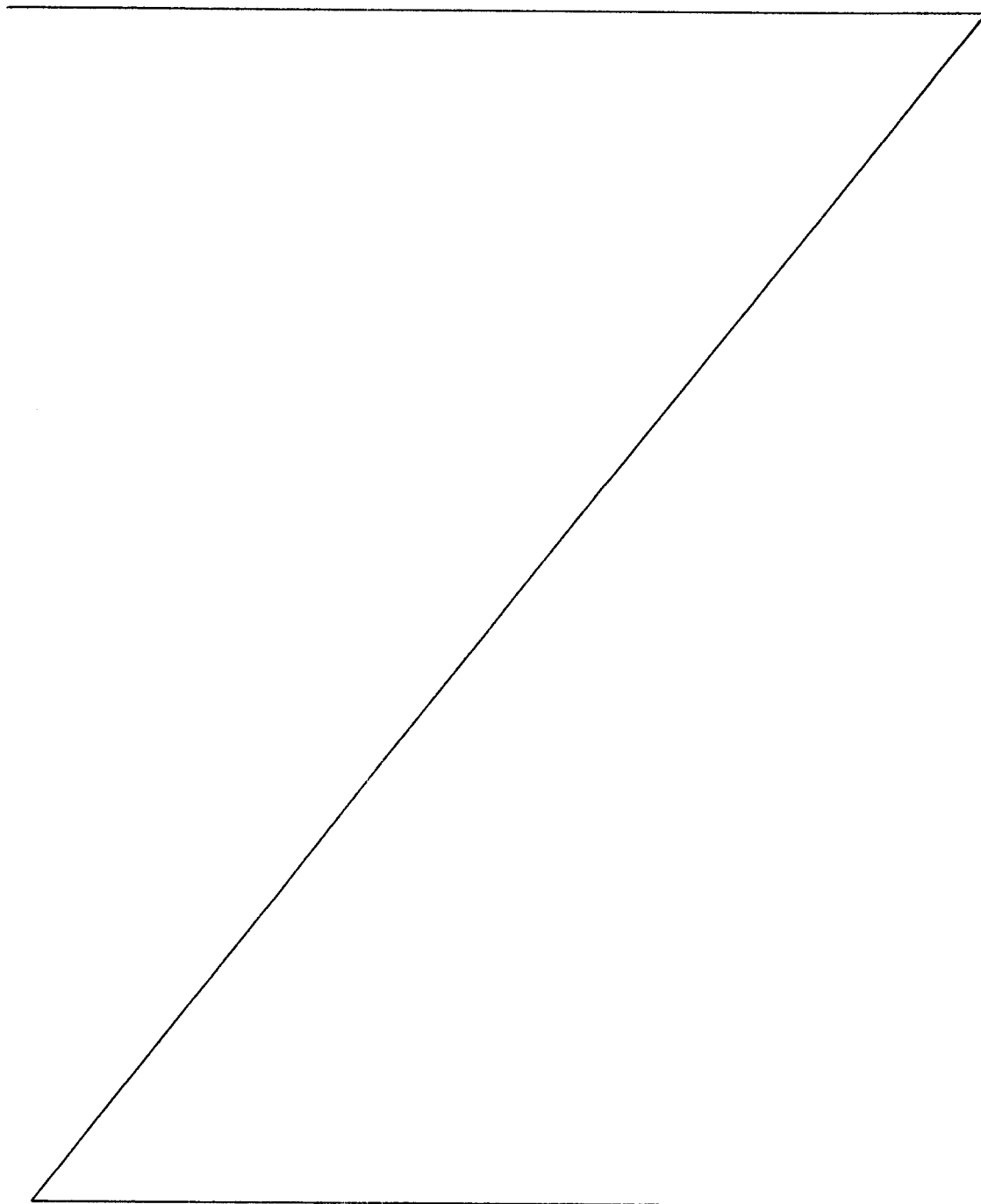
The primary physiological function of PARP 1 appears to be its involvement in a complex repair mechanism which cells have developed to repair DNA strand breaks. The primary cellular response to a DNA strand break appears moreover to consist of PARP1-catalyzed synthesis of poly(ADP-ribose) from NAD⁺ (cf. De Murcia, G. et al. (1994) TIBS, 19, 172).

PARP 1 has a modular molecular structure. Three main functional elements have been identified to date: an N-terminal 46 kD DNA binding domain; a central 22 kD automodification domain to which poly(ADP-ribose) becomes attached, with the PARP 1 enzyme activity decreasing with increasing elongation; and a C-terminal 54 kD NAD⁺ binding domain. A leucine zipper region has been found within the automodification domain, indicating possible

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protein-protein interactions, only in the PARP from *Drosophila*. All PARPs known to date are presumably active as homodimers.

The high degree of organization of the molecule is reflected in the strong conservation of the amino acid sequence. Thus, 62% conservation of the amino acid sequence has been found for PARP 1



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from humans, mice, cattle and chickens. There are greater structural differences from the PARP from *Drosophila*. The individual domains themselves in turn have clusters of increased conservation. Thus, the DNA binding region contains two so-called
5 zinc fingers as subdomains (comprising motifs of the type $CX_2CX_{28/30}HX_2C$), which are involved in the Zn^{2+} -dependent recognition of DNA single strand breaks or single-stranded DNA overhangs (e.g. at the chromosome ends, the telomeres). The C-terminal catalytic domain comprises a block of about 50 amino
10 acids (residues 859-908), which is about 100% conserved among vertebrates (PARP "signature"). This block binds the natural substrate NAD^+ and thus governs the synthesis of poly(ADP-ribose) (cf. de Murcia, loc.cit.). The GX_3GKG motif in particular is characteristic of PARPs in this block.

15

The beneficial function described above contrasts with a pathological one in numerous diseases (stroke, myocardial infarct, sepsis etc.). PARP is involved in cell death resulting from ischemia of the brain (Choi, D.W., (1997) *Nature Medicine*,
20 3, 10, 1073), of the myocardium (Zingarelli, B., et al (1997), *Cardiovascular Research*, 36, 205) and of the eye (Lam, T.T. (1997), *Res. Comm. in Molecular Pathology and Pharmacology*, 95, 3, 241). PARP activation induced by inflammatory mediators has also been observed in septic shock (Szabo, C., et al. (1997),
25 *Journal of Clinical Investigation*, 100, 3, 723). In these cases, activation of PARP is accompanied by extensive consumption of NAD^+ . Since four moles of ATP are consumed for the biosynthesis of one mole of NAD^+ , the cellular energy supply decreases drastically. The consequence is cell death.

30

PARP1 inhibitors described in the abovementioned specialist literature are nicotinamide and 3-aminobenzamide. 3,4-Di-hydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolone is disclosed
by Takahashi, K., et al (1997), *Journal of Cerebral Blood Flow*
35 and *Metabolism* 17, 1137. Further inhibitors are described, for example, in Banasik, M., et al. (1992) *J. Biol. Chem.*, 267, 3, 1569 and Griffin, R.J., et al. (1995), *Anti-Cancer Drug Design*, 10, 507.

40 High molecular weight binding partners described for human PARP1 include the base excision repair (BER) protein XRCC1 (X-ray repair cross-complementing 1) which binds via a zinc finger motif and a BRCT (BRCA1 C-terminus) module (amino acids 372-524) (Masson, M., et al., (1998) *Molecular and Cellular Biology*, 18,6,
45 3563).

It is an object of the present invention, because of the diverse physiological and pathological functions of PARP, to provide novel PARP homologs. The reason for this is that the provision of homologous PARPs would be particularly important for developing novel targets for drugs, and novel drugs, in order to improve diagnosis and/or therapy of pathological states in which PARP, PARP homologs or substances derived therefrom are involved.

10 We have found that this object is achieved by providing PARP homologs, preferably derived from human and non-human mammals, having an amino acid sequence which has

- a) a functional NAD⁺ binding domain, i.e. a PARP "signature" sequence having the characteristic GX₃GKG motif;
and
- b) especially in the N-terminal sequence region, i.e. in the region of the first 200, such as, for example, in the region of the first 100, N-terminal amino acids, no PARP zinc finger sequence motifs of the general formula



20 in which

m is an integral value from 28 or 30, and the X radicals are, independently of one another, any amino acid;
and the functional equivalents thereof.

More specifically, the present invention concerns a poly-(ADP-ribose)-polymerase (PARP) homologue or a functional equivalent thereof which is at least 85% homologous thereto, characterized by an amino acid sequence comprising

- a) a functional NAD⁺ binding domain and
- b) no zinc finger sequence motif of the general formula

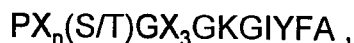
30 $CX_2CX_mHX_2C$,

wherein

m is an integer of 28 or 30 and

X is, independently at each occurrence, any amino acid residue;

wherein the functional NAD⁺ binding domain comprises the motif



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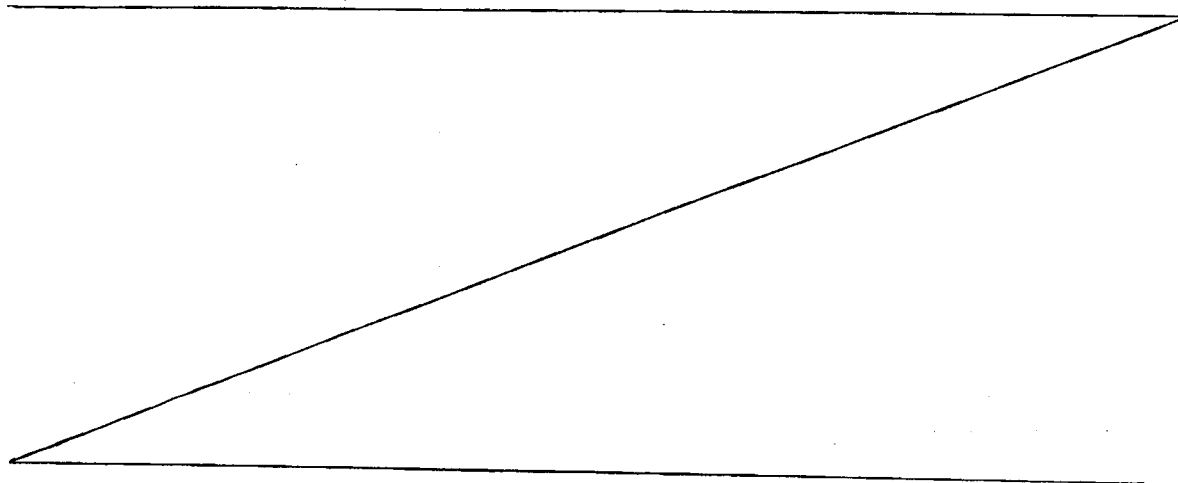
wherein

n is an integer from 1 to 5 and

X is, independently at each occurrence, any amino acid residue.

Since the PARP molecules according to the invention represent in particular functional homologs, they naturally also have a poly(ADP-ribose)-synthesizing activity. The NAD binding domain essentially corresponds to this activity and is localized to the C terminus.

- 10 Thus an essential characteristic of the PARPs according to the invention is the presence of a functional NAD⁺ binding domain (PARP signature) which is located in the C-terminal region of the amino acid sequence (i.e. approximately in the region of the last 400, such as, for example, the last 350 or 300, C-terminal amino acids), in combination with an N-terminal sequence having no zinc finger motifs. Since the zinc finger motifs in known PARPs presumably contribute to recognition of the DNA breakages, it is to be assumed that the proteins according to the invention do not interact with DNA or do so in another way. It has been
- 20 demonstrated by appropriate biochemical tests that the PARP2 according to the invention can be activated by 'activated DNA' (i.e. DNA after limited DNaseI digestion). It can be concluded from this further that the PARP2 according to the invention has DNA binding properties. However, the mechanism of the DNA binding and enzyme activation differs between the PARPs according to the invention and PARP1. Its DNA binding and enzyme activation is, as



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mentioned, mediated by a characteristic zinc finger motif. No such motifs are present in the PARPs according to the invention. Presumably these properties are mediated by positively charged amino acids in the N-terminal region of the PARPs according to 5 the invention. Since the 'activated DNA' (i.e. for example DNA after limited treatment with DNaseI) has a large number of defects (single strand breaks, single strand gaps, single-stranded overhangs, double strand breaks etc.), it is possible that although PARP1 and the PARPs according to the 10 invention are activated by the same 'activated DNA', it is by a different subpopulation of defects (e.g. single strand gaps instead of single strand breaks).

The functional NAD⁺ binding domain (i.e. catalytic domain) binds 15 the substrate for poly-(ADP-ribose) synthesis. Consistent with known PARPs, the sequence motif GX¹X²X³GKG, in which G is glycine, K is lysine, and X¹, X² and X³ are, independently of one another, any amino acid, is present in particular. However, as shown, surprisingly, by comparison of the amino acid sequences of the 20 NAD⁺ binding domains of PARP molecules according to the invention with previously disclosed human PARP1, the sequences according to the invention differ markedly from the known sequence for the NAD⁺ binding domain.

25 A group of PARP molecules which is preferred according to the invention preferably has the following general sequence motif in the catalytic domain in common:

30 PX_n(S/T)GX₃GKGIYFA (SEQ ID NO:11), in particular
(S/T)XGLR(I/V)XPX_n(S/T)GX₃GKGIYFA (SEQ ID NO:12),
preferably
LLWHG(S/T)X₇IL(S/T)XGLR(I/V)XPX_n(S/T)GX₃GKGIYFAX₃SKSAXY
(SEQ ID NO:13)

35 in which (S/T) describes the alternative occupation of this sequence position by S or T, (I/V) describes the alternative occupation of this sequence position by I or V, and n is an integral value from 1 to 5, and the X radicals are, independently of one another, any amino acid. The last motif is also referred 40 to as the "PARP signature" motif.

The automodification domain is preferably likewise present in the PARPs according to the invention. It can be located, for example, in the region from about 100 to 200 amino acids in front of the 45 N-terminal end of the NAD⁺ binding domain.

5

PARP homologs according to the invention may additionally comprise, N-terminally of the NAD⁺ binding domain (i.e. about 30 to about 80 amino acids closer to the N terminus), a leucine zipper-like sequence motif of the general formula

5 (L/V)X₆LX₆LX₆L (SEQ ID NO:14)

in which

(L/V) represents the alternative occupation of this sequence position by L or V, and the X radicals are, independently of one another, any amino acid. The leucine zipper motifs observed
10 according to the invention differ distinctly in position from those described for PARP from Drosophila. Leucine zippers may lead to homodimers (two PARP molecules) or heterodimers (one PARP molecule with a binding partner differing therefrom).

15 The PARP homologs according to the invention preferably additionally comprise, N-terminally of the abovementioned leucine zipper-like sequence motifs, i.e. about 10 to 250 amino acid residues closer to the N terminus, at least another one of the following part-sequence motifs:

20

LX₉NX₂YX₂QLLX(D/E)X_bWGRVG, (motif 1; SEQ ID NO:15)

AX₃FXKX₄KTXNXWX₅FX₃PXK, (motif 2; SEQ ID NO:16)

QXL(I/L)X₂IX₉MX₁₀PLGKLX₃QIX₆L, (motif 3; SEQ ID NO:17)

FYTXIPHXFGX₃PP, (motif 4; SEQ ID NO:18)

25

and

KX₃LX₂LXDIEXAX₂L (motif 5; SEQ ID NO:19),

in which (D/E) describes the alternative occupation of this sequence position by D or E, (I/L) describes the alternative
30 occupation of this sequence position by I or L, b is the integral value 10 or 11, and the X radicals are, independently of one another, any amino acid. It is most preferred for these motifs 1 to 5 all to be present in the stated sequence, with motif 1 being closest to the N terminus.

35

The abovementioned PARP signature motif is followed in the proteins according to the invention by at least another one of the following motifs:

40 GX₃LXEVALG (motif 6; SEQ ID NO:20)
GX₂SX₄GX₃PX_aLXGX₂V (motif 7; SEQ ID NO:21) and
E(Y/F)X₂YX₃QX₄YLL (motif 8; SEQ ID NO:22)

in which (Y/F) describes the alternative occupation of this sequence position by Y or F, a is equal to 7 to 9 and X is in
45 each case any amino acid. It is most preferred for the three

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C-terminal motifs all to be present and in the stated sequence, with motif 8 being closest to the C terminus.

A preferred PARP structure according to the invention may be described schematically as follows:

Motifs 1 to 5/PARP signature/motifs 6 to 8 or
motifs 1 to 5/leucine zipper/PARP signature/motifs 6 to 8

- 10 it being possible for further amino acid residues, such as, for example, up to 40, to be arranged between the individual motifs and for further amino acid residues, such as, for example, up to 80, to be arranged at the N terminus and/or at the C terminus.
- 15 PARP homologs which are particularly preferred according to the invention are the proteins human PARP2, human PARP3, mouse PARP3 and the functional equivalents thereof. The protein referred to as human PARP2 comprises 570 amino acids (cf. SEQ ID NO:2). The protein referred to as human PARP3 possibly exists in two forms.
- 20 Type 1 comprises 533 amino acids (SEQ ID NO:4) and type 2 comprises 540 amino acids (SEQ ID NO:6). The forms may arise through different initiation of translation. The protein referred to as mouse PARP3 exists in two forms which differ from one another by a deletion of 5 amino acids (15 bp). Type 1 comprises
- 25 533 amino acids (SEQ ID NO: 8) and type 2 comprises 528 amino acids (SEQ ID NO:10). The PARP-homologs of the present invention differ in their sequences significantly over said PARP protein of *Arabidopsis thaliana* (see above). For example, PARP2 and PARP3 do not comprise the plant PARP specific peptide sequence AAVLDQWIPD,
- 30 corresponding to amino acid residues 143 to 152 of the *Arabidopsis* protein.

- The invention further relates to the binding partners for the PARP homologs according to the invention. These binding partners
- 35 are preferably selected from
- a) antibodies and fragments such as, for example, Fv, Fab, F(ab')₂, thereof
 - b) protein-like compounds which interact, for example via the above leucine zipper region or another sequence section, with
 - 40 PARP, and
 - c) low molecular weight effectors which modulate a biological PARP function such as, for example, the catalytic PARP activity, i.e. NAD⁺-consuming ADP ribosylation, or the
 - 45 binding to an activator protein or to DNA.

The invention further relates to nucleic acids comprising

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- a) a nucleotide sequence coding for at least one PARP homolog according to the invention, or the complementary nucleotide sequence thereof;
- b) a nucleotide sequence which hybridizes with a sequence as specified in a), preferably under stringent conditions; or
- 5 c) nucleotide sequences which are derived from the nucleotide sequences defined in a) and b) through the degeneracy of the genetic code.
- 10 Nucleic acids which are suitable according to the invention comprise in particular at least one of the partial sequences which code for the abovementioned amino acid sequence motifs.

Nucleic acids which are preferred according to the invention

15 comprise nucleotide sequences as shown in SEQ ID NO: 1 and 3, and, in particular, partial sequences thereof which are characteristic of PARP homologs according to the invention, such as, for example, nucleotide sequences comprising

- 20 a) nucleotides +3 to +1715 shown in SEQ ID NO:1;
- b) nucleotides +242 to +1843 shown in SEQ ID NO:3;
- c) nucleotides +221 to +1843 shown in SEQ ID NO:5;
- d) nucleotides +112 to +1710 shown in SEQ ID NO:7; or
- e) nucleotides +1 to +1584 shown in SEQ ID NO:9

25 or partial sequences of a), b), c), d) and e) which code for the abovementioned characteristic amino acid sequence motifs of the PARP homologs according to the invention.

- 30 The invention further relates to expression cassettes which comprise at least one of the above-described nucleotide sequences according to the invention under the genetic control of regulatory nucleotide sequences. These can be used to prepare recombinant vectors according to the invention, such as, for
- 35 example, viral vectors or plasmids, which comprise at least one expression cassette according to the invention.

Recombinant microorganisms according to the invention are transformed with at least one of the abovementioned vectors.

40 The invention also relates to transgenic mammals transfected with a vector according to the invention.

The invention further relates to an in vitro detection method,

45 which can be carried out homogeneously or heterogeneously, for PARP inhibitors, which comprises

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- a) incubating an unsupported or supported poly-ADP-ribosylatable target with a reaction mixture comprising
 - a1) a PARP homolog according to the invention;
 - a2) a PARP activator; and
 - 5 a3) a PARP inhibitor or an analyte in which at least one PARP inhibitor is suspected;
- b) carrying out the polyADP ribosylation reaction; and
- c) determining the polyADP ribosylation of the target qualitatively or quantitatively.

10

The detection method is preferably carried out by preincubating the PARP homolog with the PARP activator and the PARP inhibitor or an analyte in which at least one PARP inhibitor is suspected, for example for about 1-30 minutes, before carrying out the poly-ADP ribosylation reaction.

After activation by DNA with single strand breaks (referred to as "activated DNA" according to the invention), PARP poly-ADP ribosylates a large number of nuclear proteins in the presence of NAD. These proteins include, on the one hand, PARP itself, but also histones etc.

The poly-ADP-ribosylatable target preferably used in the detection method is a histone protein in its native form or a poly-ADP-ribosylatable equivalent derived therefrom. A histone preparation supplied by Sigma (SIGMA, catalogue No. H-7755; histone type II-AS from calf thymus, Luck, J. M., et al., J. Biol. Chem., 233, 1407 (1958), Satake K., et al., J. Biol. Chem, 235, 2801 (1960)) was used by way of example. It is possible in principle to use all types of proteins or parts thereof amenable to poly-ADP-ribosylation by PARP. These are preferably nuclear proteins, e.g. histones, DNA polymerase, telomerase or PARP itself. Synthetic peptides derived from the corresponding proteins can also act as target.

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In the ELISA according to the invention it is possible to use amounts of histones in the range from about 0.1 µg/well to about 100 µg/well, preferably about 1 µg/well to about 10 µg/well. The amounts of the PARP enzyme are in a range from about 0.2 pmol/well to about 2 nmol/well, preferably from about 2 pmol/well to about 200 pmol/well, the reaction mixture comprising in each case 100 µg/well. Reductions to smaller wells and correspondingly smaller reaction volumes are possible.

45 In the HTRF assay according to the invention, identical amounts of PARP are employed, and the amount of histone or modified histones is in the range from about 2 ng/well to about 25 µg/well,

preferably about 25 ng/well to about 2.5 µg/well, the reaction mixture comprising in each case 50 µl/well. Reductions to smaller wells and correspondingly smaller reaction volumes are possible.

- 5 The PARP activator used according to the invention is preferably activated DNA.

Various types of damaged DNA can function as activator. DNA damage can be produced by digestion with DNases or other DNA-modifying enzymes (e.g. restriction endonucleases), by irradiation or other physical methods or chemical treatment of the DNA. It is further possible to simulate the DNA damage situation in a targeted manner using synthetic oligonucleotides. In the assays indicated by way of example, activated DNA from calf thymus was employed (Sigma, product No. D4522; CAS: 91080-16-9, prepared by the method of Aposhian and Kornberg using calf thymus DNA (SIGMA D-1501) and deoxyribonuclease type I (D-4263). Aposhian H. V. and Kornberg A., J. Biol. Chem., 237, 519 (1962)). The activated DNA was used in a concentration range from 0.1 to 1000 µg/ml, preferably from 1 to 100 µg/ml, in the reaction step.

The polyADP ribosylation reaction is started in the method according to the invention by adding NAD⁺. The NAD concentrations were in a range from about 0.1 µM to about 10 mM, preferably in a range from about 10 µM to about 1 mM.

In the variant of the above method which can be carried out heterogeneously, the polyADP ribosylation of the supported target is determined using anti-poly(ADP-ribose) antibodies. To do this, the reaction mixture is separated from the supported target, washed and incubated with the antibody. This antibody can itself be labeled. However, as an alternative for detecting bound anti-poly(ADP-ribose) antibody a labeled secondary antibody or a corresponding labeled antibody fragment may be applied. Suitable labels are, for example, radiolabeling, chromophore- or fluorophore-labeling, biotinylation, chemiluminescence labeling, labeling with paramagnetic material or, in particular, enzyme labels, e.g. with horseradish peroxidase. Appropriate detection techniques are generally known to the skilled worker.

40 In the variant of the above process which can be carried out homogeneously, the unsupported target is labeled with an acceptor fluorophore. The target preferably used in this case is biotinylated histone, the acceptor fluorophore being coupled via avidin or streptavidin to the biotin groups of the histone. Particularly suitable as acceptor fluorophore are phycobiliproteins (e.g. phycocyanins, phycoerythrins), e.g. R-phycocyanin (R-PC), allophycocyanin.

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cyanin (APC), R-phycoerythrin (R-PE), C-phycoerythrin (C-PC), B-phycoerythrin (B-PE) or their combinations with one another or with fluorescent dyes such as Cy5, Cy7 or Texas Red (Tandem system) (Thammapalerd, N. et al., Southeast Asian Journal of Tropical Medicine & Public Health, 27(2): 297-303 (1996); Kronick, M. N. et al., Clinical Chemistry, 29(9), 1582-1586 (1986); Hicks, J. M., Human Pathology, 15(2), 112-116 (1984)). The dye XL665 used in the examples is a crosslinked allophycocyanin (Glazer, A. N., Rev. Microbiol., 36, 173-198 (1982); Kronick, M. N., J. Imm. Meth., 92, 1-13 (1986); MacColl, R. et al., Phycobiliproteins, CRC Press, Inc., Boca Raton, Florida (1987); MacColl, R. et al., Arch. Biochem. Biophys., 208(1), 42-48 (1981)).

It is additionally preferred in the homogeneous method to determine the polyADP ribosylation of the unsupported target using anti-poly(ADP-ribose) antibody which is labeled with a donor fluorophore which is able to transfer energy to the acceptor fluorophore when donor and acceptor are close in space owing to binding of the labeled antibody to the polyADP-ribosylated histone. A europium cryptate is preferably used as donor fluorophore for the anti-poly(ADP-ribose) antibody.

Besides the europium cryptate used, other compounds are also possible as potential donor molecules. This may entail, on the one hand, modification of the cryptate cage. Replacement of the europium by other rare earth metals such as terbium is also conceivable. It is crucial that the fluorescence has a long duration to guarantee the time delay (Lopez, E. et al., Clin. Chem. 39/2, 196-201 (1993); US Patent 5,534,622).

The detection methods described above are based on the principle that there is a correlation between the PARP activity and the amount of ADP-ribose polymers formed on the histones. The assay described herein makes it possible to quantify the ADP-ribose polymers using specific antibodies in the form of an ELISA and an HTRF (homogenous time-resolved fluorescence) assay. Specific embodiments of these two assays are described in detail in the following examples.

The developed HTRF (homogeneous time-resolved fluorescence) assay system measures the formation of poly(ADP-ribose) on histones using specific antibodies. In contrast to the ELISA, this assay is carried out in homogeneous phase without separation and washing steps. This makes a higher sample throughput and a smaller susceptibility to errors possible. HTRF is based on the fluorescence resonance energy transfer (FRET) between two fluorophores. In a FRET assay, an excited donor fluorophore can

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transfer its energy to an acceptor fluorophore when the two are close to one another in space. In HTRF technology, the donor fluorophore is a europium cryptate [(Eu)K] and the acceptor is XL665, a stabilized allophycocyanin. The europium cryptate is
5 based on studies by Jean Marie Lehn (Strasbourg) (Lopez, E. et al., Clin. Chem. 39/2, 196-201 (1993); US Patent 5,534,622).

In a homogeneous assay, all the components are also present during the measurement. Whereas this has advantages for carrying out
10 the assay (rapidity, complexity), it is necessary to preclude interference by assay components (inherent fluorescence, quenching by dyes etc.). HTRF precludes such interference by time-delayed measurement at two wavelengths (665 nm, 620 nm). The HTRF has a very long decay time and time-delayed measurement is therefore
15 possible. There is no longer any interference from short-lived background fluorescence (e.g. from assay components or inhibitors of the substance library). In addition, measurement is always carried out at two wavelengths in order to compensate for quench effects of colored substances. HTRF assays can be carried out,
20 for example, in 96- or 384-well microtiter plate format and are evaluated using a discovery HTRF microplate analyzer (Canberra Packard).

Also provided according to the invention are the following in
25 vitro screening methods for binding partners for PARP, in particular for a PARP homolog according to the invention.

A first variant is carried out by

- a1) immobilizing at least one PARP homolog on a support;
- 30 b1) contacting the immobilized PARP homolog with an analyte in which at least one binding partner is suspected; and
- c1) determining, where appropriate after an incubation period, analyte constituents bound to the immobilized PARP homolog.

35 A second variant entails

- a2) immobilizing on a support an analyte which comprises at least one possible binding partner for the PARP homolog;
- b2) contacting the immobilized analyte with at least one PARP homolog for which a binding partner is sought; and
- 40 c3) examining the immobilized analyte, where appropriate after an incubation period, for binding of the PARP homolog.

The invention also relates to a method for the qualitative or quantitative determination of a nucleic acid encoding a PARP
45 homolog, which comprises

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- a) incubating a biological sample with a defined amount of an exogenous nucleic acid according to the invention (e.g. with a length of about 20 to 500 bases or longer), hybridizing, preferably under stringent conditions, determining the hybridizing nucleic acids and, where appropriate, comparing with a standard; or
- b) incubating a biological sample with a defined amount of oligonucleotide primer pairs with specificity for a PARP homolog-encoding nucleic acid, amplifying the nucleic acid, determining the amplification product and, where appropriate, comparing with a standard.

The invention further relates to a method for the qualitative or quantitative determination of a PARP homolog according to the invention, which comprises

- a) incubating a biological sample with at least one binding partner specific for a PARP homolog,
- b) detecting the binding partner/PARP complex and, where appropriate,
- c) comparing the result with a standard.

The binding partner in this case is preferably an anti-PARP antibody or a binding fragment thereof, which carries a detectable label where appropriate.

The determination methods according to the invention for PARP, in particular for PARP homologs and for the coding nucleic acid sequences thereof, are suitable and advantageous for diagnosing sepsis- or ischemia-related tissue damage, in particular strokes, myocardial infarcts, diabetes or septic shock.

The invention further comprises a method for determining the efficacy of PARP effectors, which comprises

- a) incubating a PARP homolog according to the invention with an analyte which comprises an effector of a physiological or pathological PARP activity; removing the effector again where appropriate; and
- b) determining the activity of the PARP homolog, where appropriate after adding substrates or cosubstrates.

The invention further relates to gene therapy compositions which comprise in a vehicle acceptable for gene therapy a nucleic acid construct which

- a) comprises an antisense nucleic acid against a coding nucleic acid according to the invention; or

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- b) a ribozyme against a noncoding nucleic acid according to the invention; or
- c) codes for a specific PARP inhibitor.

5 The invention further relates to pharmaceutical compositions comprising, in a pharmaceutically acceptable vehicle, at least one PARP protein according to the invention, at least one PARP binding partner according to the invention or at least one coding nucleotide sequence according to the invention.

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Finally, the invention relates to the use of binding partners of a PARP homolog for the diagnosis or therapy of pathological states in the development and/or progress of which at least one PARP protein, in particular a PARP homolog according to the

15 invention, or a polypeptide derived therefrom, is involved. The binding partner used can be, for example, a low molecular weight binding partner whose molecular weight can be, for example, less than about 2000 dalton or less than about 1000 dalton.

20 The invention additionally relates to the use of PARP binding partners for the diagnosis or therapy of pathological states mediated by an energy deficit. An energy deficit for the purpose of the present invention is, in particular, a cellular energy deficit which is to be observed in the unwell patient systemically or

25 in individual body regions, organs or organ regions, or tissues or tissue regions. This is characterized by an NAD and/or ATP depletion going beyond (above or below) the physiological range of variation of the NAD and/or ATP level and mediated preferably by a protein with PARP activity, in particular a PARP homolog according to the invention, or a polypeptide derived therefrom.

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"Energy deficit-mediated disorders" for the purpose of the invention additionally comprise those in which tissue damage is attributable to cell death resulting from necrosis or apoptosis. The

35 methods according to the invention are suitable for treating and preventing tissue damage resulting from cell damage due to apoptosis or necrosis; damage to nerve tissue due to ischemias and/or reperfusion; neurological disorders; neurodegenerative disorders; vascular stroke; for treating and preventing cardiovascular

40 disorders; for treating other disorders or conditions such as, for example, age-related macular degeneration, AIDS or other immunodeficiency disorders; arthritis; atherosclerosis; cachexia; cancer; degenerative disorders of the skeletal muscles; diabetes; cranial trauma; inflammatory disorders of the gastrointestinal

45 tract such as, for example, Crohn's disease; muscular dystrophy; osteoarthritis; osteoporosis; chronic and/or acute pain; kidney failure; retinal ischemia; septic shock (such as, for example,

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endotoxin shock); aging of the skin or aging in general; general manifestations of aging. The methods according to the invention can additionally be employed for extending the life and the proliferative capacity of body cells and for sensitizing tumor cells in connection with irradiation therapy.

The invention particularly relates to the use of a PARP binding partner as defined above for the diagnosis or therapy (acute or prophylactic) of pathological states mediated by energy deficits and selected from neurodegenerative disorders, or tissue damage caused by sepsis or ischemia, in particular of neurotoxic disturbances, strokes, myocardial infarcts, damage during or after infarct lysis (e.g. with TPA, Reteplase or mechanically with laser or Rotablator) and of microinfarcts during and after heart valve replacement, aneurysm resections and heart transplants, trauma to the head and spinal cord, infarcts of the kidney (acute kidney failure, acute renal insufficiency or damage during and after kidney transplant), damages of skeletal muscle, infarcts of the liver (liver failure, damage during or after a liver transplant), peripheral neuropathies, AIDS dementia, septic shock, diabetes, neurodegenerative disorders occurring after ischemia, trauma (craniocerebral trauma), massive bleeding, subarachnoid hemorrhages and stroke, as well as neurodegenerative disorders like Alzheimer's disease, multi-infarct dementia, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, epilepsy, especially of generalized epileptic seizures such as petit mal and tonoclonic seizures and partial epileptic seizures, such as temporal lobe, and complex partial seizures, kidney failure, also in the chemotherapy of tumors and prevention of metastasis and for the treatment of inflammations and rheumatic disorders, e.g. of rheumatoid arthritis; further for the treatment of revascularization of critically narrowed coronary arteries and critically narrowed peripheral arteries, e.g. leg arteries.

"Ischemia" comprises for the purposes of the invention a localized undersupply of oxygen to a tissue, caused by blockage of arterial blood flow. Global ischemia occurs when the blood flow to the entire brain is interrupted for a limited period. This may be caused, for example, by cardiac arrest. Focal ischemia occurs when part of the brain is cut off from its normal blood supply. Focal ischemia may be caused by thromboembolic closure of a blood vessel, by cerebral trauma, edemas or brain tumor. Even transient ischemias can lead to wideranging neuronal damage. Although damage to "nerve tissue" may occur days or weeks after the start of the ischemia, some permanent damage (e.g. necrotic cell death) occurs in the first few minutes after interruption of the blood supply. This damage is caused, for example, by the neurotoxicity

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of glutamate and follows secondary reperfusion, such as, for example, release of free radicals (e.g. oxygen free radicals, NO free radicals). Ischemias may likewise occur in other organs and tissues such as, for example, in the heart (myocardial infarct
5 and other cardiovascular disorders caused by occlusion of the coronary arteries) or in the eye (ischemia of the retina).

The invention additionally relates to the use of an effective therapeutic amount of a PARP binding partner for influencing neuronal activity. "Neuronal activity" for the purposes of the invention may consist of stimulation of damaged neurons, promotion
10 of neuronal regeneration or treatment of neuronal disorders.

"Neuronal damage" for the purposes of the invention comprises
15 every type of damage to "nerve tissue" and every physical or mental impairment or death resulting from this damage. The cause of the damage may be, for example, metabolic, toxic, chemical or thermal in nature and includes by way of example ischemias, hypoxias, trauma, cerebrovascular damage, operations, pressure, hemorrhages, irradiation, vasospasms, neurodegenerative disorders,
20 infections, epilepsy, perception disorders, disturbances of glutamate metabolism and the secondary effects caused thereby.

"Nerve tissue" for the purposes of the invention comprises the
25 various components forming the nervous system, consisting of, inter alia, neurons, glia cells, astrocytes, Schwann cells, the vascular system inside and for supplying, the CNS, brain, brain stem, spinal cord, peripheral nervous system etc.

30 "Neuroprotective" for the purposes of the invention comprises the reduction, the cessation, the slowing down or the improvement of neuronal damage and the protection, the restoration and the regeneration of nerve tissue which was exposed to neuronal damage.

35 "Prevention of neurodegenerative disorders" includes the possibility of preventing, slowing down and improving neurodegenerative disorders in people for whom such a disorder has been diagnosed or who are included in appropriate risk groups for these neurodegenerative disorders. Treatments for people already suffering
40 from symptoms of these disorders are likewise meant.

"Treatment" for the purposes of the invention comprises

- 45 (i) preventing a disorder, a disturbance or a condition in people with a predisposition thereto;

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(ii) preventing a disorder, a disturbance or a condition by slowing down its advance; and

(iii) improving a disorder, a disturbance or a condition.

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Examples of "neurological disorders" which can be treated by the methods according to the invention are neuralgias (trigeminal, glossopharyngeal), myasthenia gravis, muscular dystrophies, amyotrophic lateral sclerosis (ALS), progressive muscular atrophy, peripheral neuropathies caused by poisoning (e.g. lead poisoning),
 10 Guillain-Barré syndrome, Huntington's disease, Alzheimer's disease, Parkinson's disease, or plexus disorders. The methods according to the invention are preferably suitable for treating neurological disorders selected from peripheral neuropathies caused by physical injury or illness; cranial trauma such as, for
 15 example, traumatic brain injury; physical damage to the spinal cord; stroke associated with brain damage, such as vascular stroke in conjunction with hypoxia and brain damage, and cerebral reperfusion damage; demyelinating disorders (myelopathies, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis).

The methods according to the invention can additionally be used for treating cardiovascular disorders. "Cardiovascular disorders"
 25 for the purposes of the invention comprise those which cause ischemias or are caused by ischemias or ischemia/reperfusion of the heart. Examples are coronary vessel disorders (for example atherosclerosis), angina pectoris, myocardial infarct, cardiovascular damage due to cardiac arrest or bypass operation.

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The methods according to the invention can be used for treating cancer or for sensitizing cancer cells for irradiation therapy. The term "cancer" is to be understood in the widest sense. Modulators of the proteins according to the invention can be used as
 35 "anti-cancer therapy agents". For example, the methods can be used for treating types of cancer or tumor cells, such as ACTH-producing tumors, acute lymphatic or lymphoblastic leukemia; acute or chronic lymphocytic leukemia; acute nonlymphocytic leukemia; bladder cancer; brain tumors; breast cancer; cervical carcinoma; chronic myelocytic leukemia; bowel cancer; T-zone lymphoma; endometriosis; esophageal cancer; gall bladder cancer; Ewing's sarcoma; head and neck cancer; cancer of the tongue; Hodgkin's lymphoma; Kaposi's sarcoma; renal cancer; liver cancer; lung cancer; mesothelioma; multiple myeloma; neuroblastoma; non-
 40 Hodgkin lymphoma; osteosarcoma; ovarian carcinoma; glioblastoma; mammary carcinoma; cervical carcinoma; prostate cancer; pancreatic cancer; penis cancer; retinoblastoma; skin cancer; stomach

cancer; thyroid cancer; uterine carcinoma; vaginal carcinoma; Wilm's tumor; or trophoblastoma.

"Radiosensitizer" or "irradiation sensitizer" for the purposes of the invention relates to molecules which increase the sensitivity of the cells in the body to irradiation with electromagnetic radiation (for example X-rays) or speed up this irradiation treatment. Irradiation sensitizers increase the sensitivity of cancer cells to the toxic effects of the electromagnetic radiation. Those disclosed in the literature include mitomycin C, 5-bromo-deoxyuridine and metronidazole. It is possible to use radiation with wavelengths in the range from 10^{-20} to 10 meters, preferably gamma rays (10^{-20} to 10^{-13} m), X-rays (10^{-11} to 10^{-9} m), ultraviolet radiation (10 nm to 400 nm), visible light (400 nm to 700 nm), infrared radiation (700 nm to 1 mm) and microwave radiation (1 mm to 30 cm).

Disorders which can be treated by such a therapy are, in particular, neoplastic disorders, benign or malignant tumors and cancer. The treatment of other disorders using electromagnetic radiation is likewise possible.

The present invention will now be described in more detail with reference to the appended figures. These show:

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In Figure 1 a sequence alignment of human PARP (human PARP1) and two PARPs preferred according to the invention (human PARP2, human PARP3, murine PARP3). Sequence agreements between human PARP1 and human PARP2, human PARP3 or murine PARP3 are depicted within frames. The majority sequence is indicated over the alignment. The zinc finger motifs of human PARP1 are located in the sequence sections corresponding to amino acid residues 21 to 56 and 125 to 162;

35 In Figure 2 Northern blots with various human tissues to illustrate the tissue distribution of PARP2 and PARP3 molecules according to the invention. Lane 1: brain; lane 2: heart; lane 3: skeletal muscle; lane 4: colon; lane 5: thymus; lane 6: spleen; lane 7: kidney; lane 8: liver; lane 9: intestine; lane 10: placenta; lane 11: lung; lane 12: peripheral blood leukocytes; the respective position of the size standard (kb) is indicated.

In Figure 3 a Northern blot with further various human tissues to illustrate the tissue distribution of the PARP3 molecule according to the invention. Lane 1: heart; lane 2: brain; lane 3: placenta; lane 4: lung; lane 5: liver; lane 6: skeletal muscle; lane

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7: kidney; lane 8: pancreas; the respective position of the size standard (kb) is indicated.

In Figure 4 a Western blot with various human tissues to illustrate the tissue distribution of the PARP3 molecule according to the invention at the protein level. Lane 1: heart; lane 2: lung; lane 3: liver; lane 4: spleen; lane 5: kidney; lane 6: colon; lane 7: muscle; lane 8: brain; the respective position of the size standard (kD) is indicated.

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In Figure 5 a Western blot with various human tissues to illustrate the tissue distribution of the PARP3 molecule according to the invention. Lane 1: frontal cortex; lane 2: posterior cortex; lane 3: cerebellum; lane 4: hippocampus; lane 5: olfactory bulb; lane 6: striatum; lane 7: thalamus; lane 8: midbrain; lane 9: entorhinal cortex; lane 10: pons; lane 11: medulla; lane 12: spinal cord.

In Figure 6 a diagrammatic representation of the PARP assay (ELISA)

In Figure 7 a diagrammatic representation of the PARP assay (HTRF)

Further preferred embodiments of the invention are described in the following sections.

PARP homologs and functional equivalents

Unless stated otherwise, for the purposes of the present description amino acid sequences are indicated starting with the N terminus. If the one-letter code is used for amino acids, then G is glycine, A is alanine, V is valine, L is leucine, I is isoleucine, S is serine, T is threonine, D is aspartic acid, N is asparagine, E is glutamic acid, Q is glutamine, W is tryptophan, H is histidine, R is arginine, P is proline, K is lysine, Y is tyrosine, F is phenylalanine, C is cysteine and M is methionine.

The present invention is not confined to the PARP homologs specifically described above. On the contrary, those homologs which are functional equivalents thereof are also embraced. Functional equivalents comprise both natural, such as, for example, species-specific or organ-specific, and artificially produced variants of the proteins specifically described herein. Functional equivalents according to the invention differ by addition, substitution, inversion, insertion and/or deletion of one or more amino acid residues of human PARP2 (SEQ ID NO:2),

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human PARP3 (SEQ ID NO: 4 and 6) and mouse PARP3 (SEQ ID:8 and 10), there being at least retention of the NAD-binding function of the protein mediated by a functional catalytic C-terminal domain. Likewise, the poly(ADP-ribose)-producing catalytic activity should preferably be retained. Functional equivalents also comprise where appropriate those variants in which the region similar to the leucine zipper is essentially retained.

It is moreover possible, for example, starting from the sequence for human PARP2 or human PARP3 to replace certain amino acids by those with similar physicochemical properties (bulk, basicity, hydrophobicity, etc.). It is possible, for example, for arginine residues to be replaced by lysine residues, valine residues by isoleucine residues or aspartic acid residues by glutamic acid residues. However, it is also possible for one or more amino acids to be exchanged in sequence, added or deleted, or several of these measures can be combined together. The proteins which have been modified in this way from the human PARP2 or human PARP3 sequence have at least 60%, preferably at least 75%, very particularly preferably at least 85%, homology with the starting sequence, calculated using the algorithm of Pearson and Lipman, Proc. Natl. Acad. Sci (USA) 85(8), 1988, 2444-2448.

The following homologies have been determined at the amino acid level and DNA level between human PARP1, 2 and 3 (FastA program, Pearson and Lipman, loc. cit.):

Amino acid homologies:

	Percent identity	Percent identity in PARP signature
PARP1/PARP2	41.97% (517)	86% (50)
PARP1/PARP3	33.81% (565)	53.1% (49)
PARP2/PARP3	35.20% (537)	53.1% (49)

Numbers in parentheses indicate the number of overlapping amino acids.

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DNA Homologies:

5		Percent identity in the ORF	Percent identity in PARP signature
	PARP1/PARP2	60.81% (467)	77.85% (149)
10	PARP1/PARP3	58.81% (420)	59.02% (61)
	PARP2/PARP3	60.22% (269)	86.36% (22)

Numbers in parentheses indicate the number of overlapping nucleotides.

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The polypeptides according to the invention can be classified as homologous poly(ADP-ribose) polymerases on the basis of the great similarity in the region of the catalytic domain.

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It is also essential to the invention that the novel PARP homologs do not have conventional zinc finger motifs. This means that these enzymes are not necessarily involved in DNA repair or are so in a way which differs from PARP1, but are still able to carry out their pathological mechanism (NAD⁺ consumption and thus energy consumption due to ATP consumption). The strong protein expression, particularly of PARP3, observable in the Western blot suggests a significant role in the NAD consumption. This is particularly important for drug development. Potential novel inhibitors of the polymerases according to the invention can thus inhibit the pathological functions without having adverse effects on the desired physiological properties. This was impossible with inhibitors against the PARPs known to date since there was always also inhibition of the DNA repair function. The potentially mutagenic effect of known PARP inhibitors is thus easy to understand. It is also conceivable to design PARP inhibitors so that they efficiently inhibit all PARP homologs with high affinity. In this case, a potentiated effect is conceivable where appropriate.

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The PARP homolog which is preferred according to the invention and is shown in SEQ ID NO:2 (human PARP2) can advantageously be isolated from human brain, heart, skeletal muscle, kidney and liver. The expression of human PARP2 in other tissues or organs is distinctly weaker.

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The PARP homolog which is preferred according to the invention and is shown in SEQ ID NO: 4 and 6 (human PARP3) can advantageously be isolated from human brain (in this case very preferentially from the hippocampus), heart, skeletal muscle, 5 liver or kidney. The expression of human PARP3 in other tissues or organs, such as muscle or liver, is distinctly weaker.

The skilled worker familiar with protein isolation will make use of the combination of preparative methodologies which is most 10 suitable in each case for isolating natural PARPs according to the invention from tissues or recombinantly prepared PARPs according to the invention from cell cultures. Suitable standard preparative methods are described, for example, in Cooper, T.G., Biochemische Arbeitsmethoden, published by Walter de Gruyter, 15 Berlin, New York or in Scopes, R. Protein Purification, Springer Verlag, New York, Heidelberg, Berlin.

The invention additionally relates to PARP2 and PARP3 homologs which, although they can be isolated from other eukaryotic 20 species, i.e. invertebrates or vertebrates, especially other mammals such as, for example, mice, rats, cats, dogs, pigs, sheep, cattle, horses or monkeys, or from other organs such as, for example the myocardium, have the essential structural and functional properties predetermined by the PARPs according to the 25 invention.

In particular, the human PARP2 which can be isolated from human brain, and its functional equivalents, are preferred agents for developing inhibitors of neurodegenerative diseases as for 30 example stroke. This is because it can be assumed that drug development based on PARP2 as indicator makes it possible to develop inhibitors which are optimized for use in the human brain. However, it cannot be ruled out that inhibitors developed on the basis of PARP2 can also be employed for treating 35 PARP-mediated pathological states in other organs, too (see tissue distribution of the proteins according to the invention).

PARP2 and presumably PARP3 are also, similar to PARP1, activated by damaged DNA, although by a presumably different mechanism. 40 Significance in DNA repair is conceivable. Blockade of the PARPs according to the invention would also be beneficial in indications such as cancer (e.g. in the radiosensitization of tumor patients).

45 Another essential biological property of PARPs according to the invention and their functional equivalents is to be seen in their ability to bind an interacting partner. Human PARP2 and 3 differ

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from previously disclosed PARPs from higher eukaryotes such as, in particular, mammals by having potential so-called leucine zipper motifs. This is a typical motif for protein-protein interactions. It is possible that these motifs permit modulation of PARP activity by an interacting partner. This additional structural element thus also provides a possible starting point for development of PARP effectors such as, for example, inhibitors.

- 10 The invention thus further relates to proteins which interact with PARP2 and/or 3, preferably those which bring about their activation or inactivation.

The invention further relates to proteins which still have the abovementioned ligand-binding activity and which can be prepared starting from the specifically disclosed amino acid sequences by targeted modifications.

- It is possible, starting from the peptide sequence of the proteins according to the invention, to generate synthetic peptides which are employed, singly or in combination, as antigens for producing polyclonal or monoclonal antibodies. It is also possible to employ the PARP protein or fragments thereof for generating antibodies. The invention thus also relates to peptide fragments of PARP proteins according to the invention which comprise characteristic partial sequences, in particular those oligo- or polypeptides which comprise at least one of the abovementioned sequence motifs. Fragments of this type can be obtained, for example, by proteolytic digestion of PARP proteins or by chemical synthesis of peptides.

Novel specific PARP2 and PARP3 binding partners

- Active and preferably selective inhibitors against the proteins according to the invention were developed using the specific assay systems described above for binding partners for PARP2 and PARP3. These inhibitors optionally are also active vis a vis PARP1.

- 40 Inhibitors provided according to the invention have a strong inhibitory activity on PARP2. The K_i values may in this case be less than about 1000 nM, such as less than about 700 nM, less than about 200 nM or less than about 30 nM, e.g. about 1 to 20 nM.

- 45 Inhibitors according to the invention may also have a surprising selectivity for PARP2. This is shown by the $K_i(\text{PARP1}) : K_i(\text{PARP2})$ ratio for such inhibitors according to the invention which is,

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for example, greater than 3 or greater than 5, as for example greater than 10 or greater than 20.

An example which should be mentioned is 4-(N-(4-hydroxyphenyl)aminomethyl)-(2H)-dihydrophthalazine-1-one. The preparation of this and other analogous compounds may be performed according to Puodzhyunas et al., Pharm. Chem. J. 1973, 7, 566 or Mazkanowa et al., Zh. Obshch. Khim., 1958, 28, 2798, or Mohamed et al., Ind. J. Chem. B., 1994, 33, 769.

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The above identified compound shows a K_i value of 113 nM for PARP2 and is eight times more selective for PARP2 than for PARP3.

Nucleic acids coding for PARP homologs:

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Unless stated otherwise, nucleotide sequences are indicated in the present description from the 5' to the 3' direction.

The invention further relates to nucleic acid sequences which code for the abovementioned proteins, in particular for those having the amino acid sequence depicted in SEQ ID NO: 2, 4, 6, 8 and 10, but without being restricted thereto. Nucleic acid sequences which can be used according to the invention also comprise allelic variants which, as described above for the amino acid sequences, are obtainable by deletion, inversion, insertion, addition and/or substitution of nucleotides, preferably of nucleotides shown in SEQ ID NO: 1, 3, 7 and 9, but with essential retention of the biological properties and the biological activity of the corresponding gene product. Nucleotide sequences which can be used are obtained, for example, by nucleotide substitutions causing silent (without alteration of the amino acid sequence) or conservative amino acid changes (exchange of amino acids of the same size, charge, polarity or solubility).

Nucleic acid sequences according to the invention also embrace functional equivalents of the genes, such as eukaryotic homologs for example from invertebrates such as *Caenorhabditis* or *Drosophila*, or vertebrates, preferably from the mammals described above. Preferred genes are those from vertebrates which code for a gene product which has the properties essential to the invention as described above.

The nucleic acids according to the invention can be obtained in a conventional way by various routes:

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- For example, a genomic or a cDNA library can be screened for DNA which codes for a PARP molecule or a part thereof. For example, a cDNA library obtained from human brain, heart or kidney can be screened with a suitable probe such as, for example, a labeled
- 5 single-stranded DNA fragment which corresponds to a partial sequence of suitable length selected from SEQ ID NO: 1 or 3, or sequence complementary thereto. For this purpose, it is possible, for example, for the DNA fragments of the library which have been transferred into a suitable cloning vector to be, after
- 10 transformation into a bacterium, plated out on agar plates. The clones can then be transferred to nitrocellulose filters and, after denaturation of the DNA, hybridized with the labeled probe. Positive clones are then isolated and characterized.
- 15 The DNA coding for PARP homologs according to the invention or partial fragments can also be synthesized chemically starting from the sequence information contained in the present application. For example, it is possible for this purpose for oligonucleotides with a length of about 100 bases to be
- 20 synthesized and sequentially ligated in a manner known per se by, for example, providing suitable terminal restriction cleavage sites.

- The nucleotide sequences according to the invention can also be
- 25 prepared with the aid of the polymerase chain reaction (PCR). For this, a target DNA such as, for example, DNA from a suitable full-length clone is hybridized with a pair of synthetic oligonucleotide primers which have a length of about 15 bases and which bind to opposite ends of the target DNA. The sequence
- 30 section lying between them is then filled in with DNA polymerase. Repetition of this cycle many times allows the target DNA to be amplified (cf. White et al.(1989), Trends Genet. 5, 185).

- The nucleic acid sequences according to the invention are also to
- 35 be understood to include truncated sequences, single-stranded DNA or RNA of the coding and noncoding, complementary DNA sequence, mRNA sequences and cDNAs derived therefrom.

- The invention further embraces nucleotide sequences hybridizing
- 40 with the above sequences under stringent conditions. Stringent hybridization conditions for the purpose of the present invention exist when the hybridizing sequences have a homology of about 70 to 100%, such as, for example about 80 to 100% or 90 to 100% (preferably in an amino acid section of at least about 40, such
- 45 as, for example, about 50, 100, 150, 200, 400 or 500 amino acids).

0050/49100/49790

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Stringent conditions for the screening of DNA, in particular cDNA banks, exist, for example, when the hybridization mixture is washed with 0.1X SSC buffer (20X SSC buffer = 3M NaCl, 0.3M sodium citrate, pH 7.0) and 0.1% SDS at a temperature of about 5 60°C.

Northern blot analyses are analyses are washed under stringent conditions with 0.1X SSC, 0.1% SDS at a temperature of about 65°C, for example.

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Nucleic acid derivatives and expression constructs:

The nucleic acid sequences are also to be understood to include derivatives such as, for example, promoter variants or
15 alternative splicing variants. The promoters operatively linked upstream of the nucleotide sequences according to the invention may moreover be modified by nucleotide addition(s) or substitution(s), inversion(s), insertion(s) and/or deletion(s), but without impairing the functionality or activity of the
20 promoters. The promoters can also have their activity increased by modifying their sequence, or be completely replaced by more effective promoters even from heterologous organisms. The promoter variants described above are used to prepare expression cassettes according to the invention.

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Specific examples of human PARP2 splicing variants which may be mentioned are:

Variant human PARP2a: Deletion of base pairs 766 to 904 (cf. SEQ
30 ID NO:1). This leads to a frame shift with a new stop codon ("TAA" corresponding to nucleotides 922 to 924 in SEQ ID NO:1).
Variant human PARP2b: Insertion of
5'- gta tgc cag gaa ggt cat ggg cca gca aaa ggg tct ctg -3'
after nucleotide 204 (SEQ ID NO:1). This extends the amino acid
35 sequence by the insertion: GMPGRSWASKRVS

Nucleic acid derivatives also mean variants whose nucleotide sequences in the region from -1 to -1000 in front of the start codon have been modified so that gene expression and/or protein
40 expression is increased.

Besides the nucleotide sequence described above, the nucleic acid constructs which can be used according to the invention comprise in functional, operative linkage one or more other regulatory
45 sequences, such as promoters, amplification signals, enhancers, polyadenylation sequences, origins of replication, reporter genes, selectable marker genes and the like. This linkage may,

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depending on the desired use, lead to an increase or decrease in gene expression.

In addition to the novel regulatory sequences, it is possible for
5 the natural regulatory sequence still to be present in front of
the actual structural genes. This natural regulation can, where
appropriate, be switched off by genetic modification, and the
expression of the genes increased or decreased. However, the gene
construct may also have a simpler structure, that is to say no
10 additional regulatory signals are inserted in front of the
structural genes, and the natural promoter with its regulation is
not deleted. Instead, the natural regulatory sequence is mutated
in such a way that regulation no longer takes place, and gene
expression is enhanced or diminished. It is also possible to
15 insert additional advantageous regulatory elements at the 3' end
of the nucleic acid sequences. The nucleic acid sequences can be
present in one or more copies in the gene construct.

Advantageous regulatory sequences for the expression method
20 according to the invention are, for example, present in promoters
such as cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, lacIq,
T7, T5, T3, gal, trc, ara, SP6, l-PR or the l-PL promoter, which
are advantageously used in Gram-negative bacteria. Other
advantageous regulatory sequences are present, for example, in
25 the Gram-positive promoters amy and SPO2, in the yeast promoters
ADC1, MFa, AC, P-60, CYC1, GAPDH or in the plant promoters
CaMV/35S, SSU, OCS, lib4, usp, STLS1, B33, nos or in the
ubiquitin or phaseolin promoter.

30 It is possible in principle to use all natural promoters with
their regulatory sequences. It is also possible and advantageous
to use synthetic promoters.

Said regulatory sequences are intended to make specific
35 expression of the nucleic acid sequences and protein expression
possible. This may mean, for example, depending on the host
organism that the gene is expressed or overexpressed only after
induction, or that it is immediately expressed and/or
overexpressed.

40 The regulatory sequences or factors may moreover preferably have
a positive influence on, and thus increase or decrease, the
expression. Thus, enhancement of the regulatory elements may
advantageously take place at the level of transcription by using
45 strong transcription signals such as promoters and/or enhancers.

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However, it is also possible to enhance translation by, for example, improving the stability of the mRNA.

Enhancers mean, for example, DNA sequences which bring about increased expression via an improved interaction between RNA polymerase and DNA.

The recombinant nucleic acid construct or gene construct is, for expression in a suitable host organism, advantageously inserted into a host-specific vector which makes optimal expression of the genes in the host possible. Vectors are well known to the skilled worker and are to be found, for example, in "Cloning Vectors" (Pouwels P. H. et al., Ed., Elsevier, Amsterdam-New York-Oxford, 1985). Apart from plasmids, vectors also mean all other vectors known to the skilled worker, such as, for example, phages, viruses, such as SV40, CMV, baculovirus and adenovirus, transposons, IS elements, phasmids, cosmids, and linear or circular DNA. These vectors may undergo autonomous replication in the host organism or chromosomal replication.

20

Expression of the constructs:

The recombinant constructs according to the invention described above are advantageously introduced into a suitable host system and are expressed. Cloning and transfection methods familiar to the skilled worker are preferably used in order to bring about expression of said nucleic acids in the particular expression system. Suitable systems are described, for example, in Current Protocols in Molecular Biology, F. Ausubel et al., ed., Wiley Interscience, New York 1997.

Suitable host organisms are in principle all organisms which make it possible to express the nucleic acids according to the invention, their allelic variants, their functional equivalents or derivatives or the recombinant nucleic acid construct. Host organisms mean, for example, bacteria, fungi, yeasts, plant or animal cells. Preferred organisms are bacteria such as those of the genera *Escherichia*, such as, for example, *Escherichia coli*, *Streptomyces*, *Bacillus* or *Pseudomonas*, eukaryotic microorganisms such as *Saccharomyces cerevisiae*, *Aspergillus*, higher eukaryotic cells from animals or plants, for example Sf9 or CHO cells.

The gene product can also, if required, be expressed in transgenic organisms such as transgenic animals such as, in particular, mice, sheep, or transgenic plants. The transgenic organisms may also be so-called knock-out animals or plants in which the corresponding endogenous gene has been switched off,

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such as, for example, by mutation or partial or complete deletion.

The combination of the host organisms and the vectors appropriate
5 for the organisms, such as plasmids, viruses or phages, such as,
for example, plasmids with the RNA polymerase/promoter system,
phages λ , μ or other temperate phages or transposons and/or other
advantageous regulatory sequences forms an expression system. The
term expression systems preferably means, for example, a
10 combination of mammalian cells such as CHO cells, and vectors,
such as pcDNA3neo vector, which are suitable for mammalian cells.

As described above, the gene product can also be expressed
advantageously in transgenic animals, e.g. mice, sheep, or
15 transgenic plants. It is likewise possible to program cell-free
translation systems with the RNA derived from the nucleic acid.

The gene product can also be expressed in the form of
therapeutically or diagnostically suitable fragments. To isolate
20 the recombinant protein it is possible and advantageous to use
vector systems or oligonucleotides which extend the cDNA by
certain nucleotide sequences and thus code for modified
polypeptides which serve to simplify purification. Suitable
modifications of this type are, for example, so-called tags which
25 act as anchors, such as, for example, the modification known as
the hexa-histidine anchor, or epitopes which can be recognized as
antigens by antibodies (described, for example, in Harlow, E. and
Lane, D., 1988, Antibodies: A Laboratory Manual. Cold Spring Har-
bor (N.Y.) Press). These anchors can be used to attach the
30 proteins to a solid support such as, for example, a polymer
matrix, which can, for example, be packed into a chromatography
column, or to a microtiter plate or to another support.

These anchors can also at the same time be used to recognize the
35 proteins. It is also possible to use for recognition of the
proteins conventional markers such as fluorescent dyes, enzyme
markers which form a detectable reaction product after reaction
with a substrate, or radioactive markers, alone or in combination
with the anchors for derivatizing the proteins.

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Production of antibodies:

Anti-PARP2 antibodies are produced in a manner familiar to the
skilled worker. Antibodies mean both polyclonal, monoclonal,
45 human or humanized antibodies or fragments thereof, single chain
antibodies or also synthetic antibodies, likewise antibody
fragments such as Fv, Fab and F(ab')₂. Suitable production methods

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are described, for example, in Campbell, A.M., Monoclonal Antibody Technology, (1987) Elsevier Verlag, Amsterdam, New York, Oxford and in Breitling, F. and Dübel, S., Rekombinante Antikörper (1997), Spektrum Akademischer Verlag, Heidelberg.

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Further use of the coding sequence:

The present cDNA additionally provides the basis for cloning the genomic sequence of the novel PARP genes. This also includes the relevant regulatory or promoter sequence, which is available, for example, by sequencing the region located 5' upstream of the cDNA according to the invention or located in the introns of the genes. The cDNA sequence information is also the basis for producing antisense molecules or ribozymes with the aid of known methods (cf. Jones, J.T. and Sallenger, B.A. (1997) Nat. Biotechnol. 15, 902; Nellen, W. and Lichtenstein, C. (1993) TIBS, 18, 419). The genomic DNA can likewise be used to produce the gene constructs described above.

20 Another possibility of using the nucleotide sequence or parts thereof is to generate transgenic animals. Transgenic overexpression or genetic knock-out of the sequence information in suitable animal models may provide further valuable information about the (patho)physiology of the novel genes.

25

Therapeutic applications:

In situations where there is a prevailing deficiency of a protein according to the invention it is possible to employ several methods for replacement. On the one hand, the protein, natural or recombinant, can be administered directly or by gene therapy in the form of its coding nucleic acid (DNA or RNA). It is possible to use any suitable vectors for this, for example both viral and non-viral vehicles. Suitable methods are described, for example, by Strauss and Barranger in Concepts in Gene Therapy (1997), Walter de Gruyter, publisher. Another alternative is provided by stimulation of the endogenous gene by suitable agents.

It is also possible to block the turnover or the inactivation of PARPs according to the invention, for example by proteases. Finally, inhibitors or agonists of PARPs according to the invention can be employed.

In situations where a PARP is present in excess or is overactivated, various types of inhibitors can be employed. This inhibition can be achieved both by antisense molecules,

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ribozymes, oligonucleotides or antibodies, and by low molecular weight compounds.

The active substances according to the invention, i.e. PARP proteins, nucleic acids and PARP binding partners such as, for example, antibodies or modulators, can be administered either as single therapeutic active substances or as mixtures with other therapeutic active substances. They can be administered as such, but in general they are administered in the form of pharmaceutical compositions, i.e. as mixtures of the active substance(s) with at least one suitable pharmaceutical carrier or diluent. The active substances or compositions can be administered in any way suitable for the particular therapeutic purpose, e.g. orally or parenterally.

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The nature of the pharmaceutical composition and of the pharmaceutical carrier or diluent depends on the required mode of administration. Oral compositions can be, for example, in the form of tablets or capsules and may contain customary excipients such as binders (e.g. sirup, acacia, gelatin, sorbitol, tragacanth or polyvinylpyrrolidone), bulking agents (e.g. lactose, sugar, corn starch, calcium phosphate, sorbitol or glycine), lubricants (e.g. magnesium stearate, talc, polyethylene glycol or silica), disintegrants (e.g. starch) or wetting agents (e.g. sodium lauryl sulfate). Oral liquid products may be in the form of aqueous or oily suspensions, solutions, emulsions, sirups, elixirs or sprays etc. or may be in the form of dry powders for reconstitution with water or another suitable carrier. Liquid products of these types may contain conventional additives, for example suspending agents, flavorings, diluents or emulsifiers. It is possible to employ for parenteral administration solutions or suspensions with conventional pharmaceutical carriers. Parenteral administration of active substances according to the invention advantageously takes place using a liquid pharmaceutical composition which can be administered parenterally, in particular intravenously. This preferably contains an effective amount of at least one active substance, preferably in dissolved form, in a pharmaceutically acceptable carrier suitable for this purpose. Examples of pharmaceutical carriers suitable for this purpose are, in particular, aqueous solutions such as, for example, physiological saline, phosphate-buffered saline, Ringer's solution, Ringer's lactate solution and the like. The composition may moreover contain further additions such as antioxidants, chelating agents or antimicrobial agents.

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The choice in each case of the dosage of the active substances according to the invention and the particular dosage schedule are subject to a decision of the treating physician. The latter will select a suitable dose and an appropriate dosage schedule depending on the chosen route of administration, on the efficacy of the medicine in each case, on the nature and severity of the disorder to be treated, and on the condition of the patient and his response to the therapy. Thus, for example, the pharmacologically active substances can be administered to a mammal (human or animal) in doses of about 0.5 mg to about 100 mg per kg of body weight and day. They can be administered in a single dose or in several doses.

Nontherapeutic applications:

The nucleic acids according to the invention, such as, for example, cDNA, the genomic DNA, the promoter, and the polypeptide, and partial fragments thereof, can also be used in recombinant or nonrecombinant form for developing various test systems.

For example, it is possible to establish a test system which is suitable for measuring the activity of the promoter or of the protein in the presence of a test substance. The methods of measurement in this case are preferably simple ones, e.g. colorimetric, luminometric, fluorimetric, immunological or radioactive, and allow preferably a large number of test substances to be measured rapidly. Tests of this type are suitable and advantageous for so-called high-throughput screening. These test systems allow test substances to be assessed for their binding to or their agonism, antagonism or inhibition of proteins according to the invention.

Determination of the amount, activity and distribution of the proteins according to the invention or their underlying mRNA in the human body can be used for the diagnosis, for the determination of the predisposition and for the monitoring of certain diseases. Likewise, the sequence of the cDNA and the genomic sequence may provide information about genetic causes of and predispositions to certain diseases. It is possible to use for this purpose both DNA/RNA probes and antibodies of a wide variety of types. The nucleotide sequences according to the invention or parts thereof can further be used in the form of suitable probes for detecting point mutations, deletions or insertions.

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The proteins according to the invention can further be used to identify and isolate their natural ligands or interacting partners. The proteins according to the invention can additionally be used to identify and isolate artificial or
5 synthetic ligands. For this purpose, the recombinantly prepared or purified natural protein can be derivatized in such a way that it has modifications which permit linkage to support materials. Proteins bound in this way can be incubated with various analytes, such as, for example, protein extracts or peptide
10 libraries or other sources of ligands. Specifically bound peptides, proteins or low molecular weight, non-proteinogenous substances can be isolated and characterized in this way. Non-proteinogenous substances mean, for example, low molecular weight chemical substances which may originate, for example, from
15 classical drug synthesis or from so-called substance libraries which have been synthesized combinatorially.

The protein extracts used are derived, for example, from homogenates of plants or parts of plants, microorganisms, human
20 or animal tissues or organs.

Ligands or interacting partners can also be identified by methods like the yeast two-hybrid system (Fields, S. and Song, O. (1989) Nature, 340, 245). The expression banks which can be employed in
25 this case may be derived, for example, from human tissues such as, for example, brain, heart, kidney etc.

The nucleic acid sequences according to the invention and the proteins encoded by them can be employed for developing reagents,
30 agonists and antagonists or inhibitors for the diagnosis and therapy of chronic and acute diseases associated with the expression or activation of one of the protein sequences according to the invention, such as, for example, with increased or decreased expression thereof. The reagents, agonists,
35 antagonists or inhibitors developed can subsequently be used to produce pharmaceutical preparations for the treatment or diagnosis of disorders. Examples of possible diseases in this connection are those of the brain, of the peripheral nervous system, of the cardiovascular system or of the eye, of septic
40 shock, of rheumatoid arthritis, diabetes, acute kidney failure, or of cancer.

The relevance of the proteins according to the invention for said indications was verified using specific inhibitors in relevant
45 animal models.

The invention is now illustrated in detail with reference to the following examples.

Example 1: Isolation of the PARP2 and PARP3 cDNA

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The present cDNA sequences were found for the first time on sequence analysis of cDNA clones of a cDNA library from human brain (Human Brain 5' Stretch Plus cDNA Library, # HL3002a, Clontech). The mouse PARP3 clones were isolated from a "lambda
10 triplex mouse brain cDNA library" (Clontech order No. ML5004t). The sequences of these clones are described in SEQ ID NO:1, 3, 7 and 9.

Example 2: Expression of PARP2 and PARP3 in human tissues

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The expression of human PARP2 and human PARP3 was investigated in twelve different human tissues by Northern blot analysis. A Human Multiple Tissue Northern Blot (MTN™) supplied by Clontech (#7760-1 and #7780-1) was hybridized for this purpose with an RNA
20 probe. The probe was produced by in vitro transcription of the corresponding cDNA of human PARP2 and human PARP3 in the presence of digoxigenin-labeled nucleotides in accordance with the manufacturer's method (BOEHRINGER MANNHEIM DIG Easy Hyb order No. 1603 558, DIG Easy Hyb method for RNA:RNA hybridization). The
25 protocol was modified to carry out the prehybridization: 2x1h with addition of herring sperm DNA (10 mg/ml of hybridization solution). Hybridization then took place overnight with addition of herring sperm DNA (10 mg/ml of hybridization solution). The bands were detected using the CDP-Star protocol (BOEHRINGER
30 MANNHEIM CDP-Star™ order No. 1685 627).

After stringent washing, the transcript of PARP2 was mainly detected in human brain, heart, skeletal muscle, kidney and liver. The transcript size of about 1.9 kb corresponds to the
35 length of the cDNA determined (1.85kb) (cf. Figure 2(A)).

In other tissues or organs, human PARP2 expression is considerably weaker.

40 After stringent washing, the transcript of PARP3 was mainly detected in heart, brain, kidney, skeletal muscle and liver. Expression in other tissues (placenta, lung, pancreas) is distinctly weaker (cf. Figure 2(B)). There are at least 2 transcripts for human PARP3, which can presumably be explained by
45 different polyadenylation sites or alternative splicing. Their size (about 2.2 kb and 2.5 kb respectively) corresponds to the length of the cDNA determined (2.3kb). Washing was carried out

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with 0.2 x SSC/0.2% SDS at room temperature for 2 x 15 minutes and then with 0.1 x SSC/0.1% SDS at 65°C for 2 x 15 minutes (prepared from 20X SSC: 3M NaCl, 0.3M sodium citrate, pH 7.0).

5 Example 3: Production of antibodies

Specific antibodies against the proteins according to the invention were produced. These were used inter alia for analyzing the tissue distribution at the protein level of PARP2 and PARP3 by
10 immunoblot (Western blot) analysis. Examples of the production of such antibodies are indicated below.

The following peptides were prepared by synthesis in the manner familiar to the skilled worker for the antibody production. In
15 some cases, a cysteine residue was attached to the N or C terminals of the sequences in order to facilitate coupling to KLH (keyhole limpet hemocyanin).

PARP-2: NH₂-MAARRRRSTGGGRARALNES-CO₂H (amino acids 1-20;
20 SEQ ID NO: 23)
NH₂-KTELQSPEHPLDQHYRNLHC-CO₂H (amino acids 335-353;
SEQ ID NO: 24)
PARP-3: NH₂-CKGRQAGREEDPFRSTAEALK-CO₂H (amino acids 25-44
SEQ ID NO: 25)
25 NH₂-CKQQIARGFEALEALEEALK-CO₂H (amino acids 230-248;
SEQ ID NO: 26)

The production of an anti-PARP3 antibody is described as a representative example.

30

For human PARP3, polyclonal antibodies were raised in rabbits using a synthetic peptide having the peptide sequence H₂N-KQQIARGFEALEALEEALK-CO₂H (SEQ ID NO: 27) (amino acids 230-248 of the human PARP3 protein sequence). The corresponding mouse sequence differs
35 in this region only by one amino acid (H₂N-KQQIARGFEALEALEEAMK-CO₂H; SEQ ID NO: 28). A cysteine was also attached to the N terminus in order to make it possible for the protein to couple to KLH.

40 Rabbits were immunized a total of five times, at intervals of 7-14 days, with the KLH-peptide conjugate. The antiserum obtained was affinity-purified using the antigen. The specific IgG fraction was isolated from the serum using the respective peptides which, for this purpose, were initially immobilized on an affinity column in the manner familiar to the skilled worker. The re-
45 spective antiserum was loaded onto this affinity column, and non-specifically sorbed proteins were eluted with buffer. The spe-

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cifically bound IgG fraction was eluted with 0.2 M glycine/HCl buffer pH 2.2. The pH was immediately increased using a 1M TRIS/HCl buffer pH 7.5. The eluate containing the IgG fraction was mixed 1:1 (volume) with saturated ammonium sulfate solution and
5 incubated at +4°C for 30 min to complete the precipitation. The resulting precipitate was centrifuged at 10,000 g and, after removal of the supernatant, dissolved in the minimum amount of PBS/TBS. The resulting solution was then dialyzed against PBS/TBS in the ratio 1:100 (volume). The antibodies were adjusted to a concentration of about 100 µg of IgG/ml. The PARP3 antibodies purified in this way had high specificity for PARP3. Whereas mouse PARP3 was recognized well, there was no observable cross-reaction with PARP1 or PARP2.

15 Example 4: Analysis of the tissue distribution by immunoblot (Western blot)

The tissue distribution at the protein level was also investigated for PARP2 and PARP3 by immunoblot (Western blot) analysis.

20

Preparation of the mouse tissues for protein gels:

Tissues or cells were homogenized using a Potter or Ultra-Turrax. For this, 0.5 g of tissue (or cells) was incubated in 5 ml of
25 buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 6 mM MgCl₂), one tablet of protease inhibitor cocktail (Boehringer Mannheim, order No.: 1836153) and benzonase (purity grade I, MERCK) at 37°C for 30 min. Tissue samples from mice were produced for heart, lung, liver, spleen, kidney, intestine, muscle, brain and for human embryonic
30 kidney cells (HEK293, human embryonal kidney).

Protein gels:

The NuPAGE system supplied by NOVEX was used according to the
35 instructions for protein gels. Polyacrylamide gels (NuPAGE 4-12% BisTris, NOVEX NP 0321), running buffer (MES-Running Buffer, NOVEX NP 0002), antioxidant (NOVEX NP 0005), protein size standard (Multi Mark Multi Colored Standard, NOVEX LC 5725), sample buffer (NuPAGE LDS Sample Buffer (4X), NOVEX NP 0007) were used.
40 The gels were run for 45 minutes at a voltage of 200 V.

Western blot:

Western blots were carried out using the NOVEX system in accordance with instructions. A nitrocellulose membrane (Nitrocellulose Pore size 45 µm, NOVEX LC 2001) was used. The transfer took 1 hour at a current of 200 mA. The transfer buffer consisted of 50 ml of

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transfer buffer concentrate (NOVEX NP 0006), 1 ml of antioxidant (NOVEX NP 0002), 100 ml of analytical grade methanol and 849 ml of double-distilled water.

- 5 Besides the blots produced in this way, also used were premade blots, for example from Chemicon (mouse brain blot, Chemicon, catalog No.: NS 106 with the tissues 1. frontal cortex, 2. posterior cortex, 3. cerebellum, 4. hippocampus, 5. olfactory bulb, 6. striatum, 7. thalamus, 8. mid brain, 9. entorhinal cortex, 10. pons, 11. medulla, 12. spinal cord).

Antibody reaction with PARP3:

- The Western blots were blocked in TBST (TBS + 0.3 % Tween* 20) with 5% dry milk powder for at least 2 hours (TBS: 100 mM Tris pH 7.5, 200 mM NaCl). The antibody reaction with the primary antibody (dilution 1:1000) took place in TBST with 5% dry milk powder (see above) at room temperature for at least 2 hours or at 4°C overnight, with gentle agitation (vertical rotator). This was followed by washing three times in TBST for 5 minutes. Incubation with the secondary antibody (anti-rabbit IgG, peroxidase-coupled, SIGMA A-6154, dilution 1:2000) took place in TBST with 5% dry milk powder for 1 hour. This was followed by washing three times for 5 minutes each time as above. The subsequent detection was based on chemiluminescence using the SUPER BLAZE kit (Pierce, Signal BLAZE Chemiluminescent Substrate 34095) as stated by the manufacturer. The "Lumi-Film" (Chemiluminescent Detection Film, Boehringer order No: 1666916) was used. The films were developed for about 2 min (X-ray developer concentrate, ADEFO-Chemie GmbH), hydrated, fixed for about 4 min (Acidofix 85 g/l /AGFA), hydrated and then dried.

Example 5: Preparation of the enzymes

- 35 For comparison, human PARP1 was expressed recombinantly in the baculovirus system in the manner familiar to the skilled worker and partially purified as described (Shah et al., Analytical Biochemistry 1995, 227, 1-13). Bovine PARP1 in a purity of 30-50% (c= 0.22 mg/ml, spec. activity 170 nmol of ADP-ribose/min/mg of total protein at 25°C) was purchased from BIOMOL (order No. SE-165). Human and mouse PARP2 and PARP3 were expressed recombinantly in the baculovirus system (Bac-to-Bac system, BRL LifeScience). For this purpose, the appropriate cDNAs were cloned to the pFASTBAC-1 vector. Preparation of recombinant baculovirus DNA by recombination in E. coli was followed by transfection of insect cells (Sf9 or High-Five) with the appropriate recombinant baculovirus DNAs. Expression of the corresponding proteins was veri-

* Trademark

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fied by Western blot analysis. Virus strains were amplified in the manner familiar to the skilled worker. Larger amounts of recombinant proteins were obtained by infecting 500 ml of insect cell culture (2×10^6 cells/ml) with viruses in an MOI (multiplicity of infection; ratio of viruses to cells) of 5-10 and incubating for 3 to 4 days. The insect cells were then pelleted by centrifugation, and the proteins were purified from the pellet.

The purification took place by classical methods of protein purification familiar to the skilled worker, detecting the enzymes with appropriate specific antibodies. In some cases, the proteins were also affinity-purified on a 3-aminobenzamide affinity column as described (Burtscher et al., Anal Biochem 1986, 152:285-290). The purity was >90%.

15

Example 6: Assay systems for determining the activity of PARP2 and PARP3 and the inhibitory action of effectors on PARP1, PARP2 and PARP3.

20 a) Production of antibodies against poly(ADP-ribose)

It is possible to use poly(ADP-ribose) as antigen for generating anti-poly(ADP-ribose) antibodies. The production of anti-poly(ADP-ribose) antibodies is described in the literature (Kanai Y et al. (1974) Biochem Biophys Res Comm 59:1, 300-306; Kawamatsu H et al. (1984) Biochemistry 23, 3771-3777; Kanai Y et al. (1978) Immunology 34, 501-508).

The following were used, inter alia: anti-poly(ADP-ribose) antibodies (polyclonal antiserum, rabbits), BIOMOL; order No. SA-276, anti-poly(ADP-ribose) antibodies (monoclonal, mouse; clone 10H; hybridoma supernatant, affinity-purified).

The antisera or monoclonal antibodies obtained from hybridoma supernatant were purified by protein A affinity chromatography in the manner familiar to the skilled worker.

b) ELISA

40 Materials:

ELISA color reagent: TMB mix, SIGMA T-8540

A 96-well microtiter plate (FALCON Micro-Test III™ Flexible Assay Plate, # 3912) was coated with histones (SIGMA, H-7755). Histones were for this purpose dissolved in carbonate buffer (0.05M Na_2HCO_3 ; pH 9.4) in a concentration of 50 $\mu\text{g}/\text{ml}$. The individual

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wells of the microtiter plate were each incubated with 150 μ l of this histone solution at room temperature for at least 2 hours or at 4°C overnight. The wells are then blocked by adding 150 μ l of a 1% BSA solution (SIGMA, A-7888) in carbonate buffer at room temperature for 2 hours. This is followed by three washing steps with washing buffer (0.05% Tween10 in 1x PBS; PBS (Phosphate buffered saline; Gibco, order No. 10010): 0.21g/l KH_2PO_4 , 9g/l NaCl, 0.726g/l $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, pH 7.4). Washing steps were all carried out in a microtiter plate washer ("Columbus" microtiter plate washer, SLT-Labinstruments, Austria).

Required for the enzyme reaction were an enzyme reaction solution and a substrate solution, in each case as a premix. The absolute amount of these solutions depended on the intended number of assay wells.

Composition of the enzyme reaction solution per well:

- 4 μ l of PARP reaction buffer (1M Tris-HCl pH 8.0, 100mM MgCl_2 , 10mM DTT)
- 20 - 20ng of PARP1 (human or bovine) or 8ng PARP2 (human or mouse)
- 4 μ l of activated DNA (1 mg/ml; SIGMA, D-4522)
- H_2O ad 40 μ l

Composition of the substrate solution per well:

- 25 - 5 μ l of PARP reaction buffer (10x)
- 0.8 μ l of NAD solution (10mM, SIGMA N-1511)
- 44 μ l H_2O

Inhibitors were dissolved in 1x PARP reaction buffer. DMSO, which was occasionally used to dissolve inhibitors in higher concentrations, was no problem up to a final concentration of 2%. For the enzyme reaction, 40 μ l of the enzyme reaction solution were introduced into each well and incubated with 10 μ l of inhibitor solution for 10 minutes. The enzyme reaction was then started by adding 50 μ l of substrate solution per well. The reaction was carried out at room temperature for 30 minutes and then stopped by washing three times with washing buffer.

The primary antibodies employed were specific anti-poly(ADP-ribose) antibodies in a dilution of 1:5000. Dilution took place in antibody buffer (1% BSA in PBS; 0.05% Tween20). The incubation time for the primary antibodies was one hour at room temperature. After subsequently washing three times with washing buffer, incubation was carried out with the secondary antibody (anti-mouse IgG, Fab fragments, peroxidase-coupled, Boehringer Mannheim, order No. 1500.686; anti-rabbit IgG, peroxidase-coupled, SIGMA, order No. A-6154) in a dilution of 1:10,000 in antibody buffer at

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room temperature for one hour. Washing three times with washing buffer was followed by the color reaction using 100 μ l of color reagent (TMB mix, SIGMA) per well at room temperature for about 15 min. The color reaction was stopped by adding 100 μ l of 2M H_2SO_4 . This was followed by immediate measurement in an ELISA plate reader (EAR340AT "Easy Reader", SLT-Labinstruments, Austria) (450nm versus 620nm). The measurement principle is depicted diagrammatically in Figure 6.

- 10 Various concentrations were used to construct a dose-effect plot to determine the K_i value of an inhibitor. Values are obtained in triplicate for a particular inhibitor concentration. Arithmetic means are determined using Microsoft® Excel. The IC_{50} is determined using the Microcal® Origin Software (Vers. 5.0) ("Sigmoidal Fit"). Conversion of the IC_{50} value is calculated in this way into K_i values took place by using "calibration inhibitors". The "calibration inhibitors" were also measured in each analysis. The K_i values of the "calibration inhibitors" were determined in the same assay system by analysis of the Dixon diagram in the manner familiar to the skilled worker.

b) HTRF (homogenous time-resolved fluorescence) assay

- In the HTRF PARP assay according to the invention, histones, as target proteins for modification by PARP, are labeled indirectly with an XL665 fluorophore. The anti poly(ADP ribose) antibody is directly labeled with a europium cryptate (anti-PAR-cryptate). If the XL665 fluorophore is in the direct vicinity in space, which is ensured by binding to the poly(ADP-ribose) on the histone, then energy transfer is possible. The emission at 665 nm is thus directly proportional to the amount of bound antibody, which in turn is equivalent to the amount of poly(ADP-ribose). The measured signal thus corresponds to the PARP activity. The measurement principle is depicted diagrammatically in Figure 7.
- 35 The materials used are identical to those used in the ELISA (see above) unless expressly indicated.

- Histones were dissolved in a concentration of 3 mg/ml in Hepes buffer (50mM, pH=7.5). Biotinylation took place with sulfo-NHS-LC-biotin (Pierce, #21335T). A molar ratio of 4 biotin molecules per histone was used. The incubation time was 90 minutes (RT). The biotinylated histones were then purified on a G25 SF HR10/10 column (Pharmacia, 17-0591-01) in Hepes buffer (50mM, pH=7.0) in order to remove excess biotinylation reagent.
- 45 The anti-poly(ADP-ribose) antibody was labeled with europium cryptate using bifunctional coupling reagents (Lopez, E. et al., Clin. Chem. 39(2), 196-201 (1993); US Patent 5,534,622).

40

Purification took place on a G25SF HR10/30 column. A molar ratio of 3.1 cryptates per antibody was achieved. The yield was 25%. The conjugates were stored at -80°C in the presence of 0.1% BSA in phosphate buffer (0.1M, pH=7).

5

For the enzyme reaction, the following were pipetted into each well:

- 10 µl of PARP solution in PARP HTRF reaction buffer (50mM Tris-HCl pH 8.0, 10mM MgCl₂, 1mM DTT) with 20ng of PARP1 (human or bovine) or 8ng of PARP2 (human or mouse)
- 10 µl of activated DNA in PARP HTRF reaction buffer (50µg/ml)
- 10 µl of biotinylated histones in PARP HTRF reaction buffer (1.25µM)
- 10 µl of inhibitor in PARP HTRF reaction buffer

15

These reagents were incubated for 2 minutes before the reaction was started by adding

- 10 µl of NAD solution in PARP HTRF reaction buffer (41 µM/ml).
- The reaction time was 30 minutes at room temperature.

20

The reaction was then stopped by adding

- 10 µl of PARP inhibitor (25 µM, K_i=10nM) in "Revelation" buffer (100mM Tris-HCl pH 7.2, 0.2M KF, 0.05% BSA).

25 The following were then added:

- 10 µl of EDTA solution (SIGMA, E-7889, 0.5M in H₂O)
- 100 µl of Sa-XL665 (Packard Instruments) in "Revelation" buffer (15-31.25nM)
- 50 µl of anti-PAR cryptate in "Revelation" buffer (1.6-3.3nM).

30

Measurement was then possible after 30 minutes (up to 4 hours). The measurement took place in a "discovery HTRF microplate analyzer" (Canberra Packard Instruments). The K_i values were calculated as described for the ELISA.

35

Example 7: Test systems for determining the therapeutic efficacy of PARP inhibitors

40 Novel PARP inhibitors can have their therapeutic efficacy checked in relevant pharmacological models. Examples of some suitable models are listed in Table 1.

45

41

	Disorder	Model	Literature
5	Neurodegenerative disorders (stroke, Parkinson's, etc.)	NMDA excitotoxicity in mice or rats	See below for description
10	Stroke	Permanent MCAO ("middle cerebral arterial occlusion")	Tokime, T. et al., J. Cereb. Blood Flow Metab., 18(9): 991-7, 1998. Guegan, C., Brain Research. Molecular Brain Research, 55(1): 133-40, 1998.
15		Transient, focal MCAO in rats or mice	Eliasson MJL et al., Nat Med 1997, 3:1089-1095. Endres, M et al., J Cereb Blood Flow Metab 1997, 17:1143-1151.
20			Takahashi K et al., J Cereb Blood Flow Metab 1997, 17:1137-1142.
25	Parkinson's disease	MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) toxicity in mice/ rats	Cosi C, et al., Brain Res., 1998 809(1):58-67. Cosi C, et al., Brain Res., 1996 729(2):264-9.
30	Myocardial infarct	Coronary vessel occlusion in rats, pigs or rabbits	Richard V, et al., Br. J. Pharmacol 1994, 113, 869-876. Thiemermann C, et al., Proc Natl Acad Sci U S A. 1997, 94(2):679-83.
35			Zingarelli B, et al., Cardiovasc Res. 1997, 36(2):205-15.
40		Langendorf heart model in rats or rabbits	See below for description
	Septic shock	Endotoxin shock in rats	Szabo C, et al., J Clin Invest, 1997, 100(3):723-35.

42

5		Zymosan- or carrageenan-induced multiple organ failure in rats or mice	Szabo C, et al. J Exp Med. 1997, 186(7):1041-9. Cuzzocrea S, et al. Eur J Pharmacol. 1998, 342(1):67-76.
	Rheumatoid arthritis	Adjuvant- or collagen-induced arthritis in rats or mice	Szabo C, et al., Proc Natl Acad Sci U S A. 1998, 95(7):3867-72.
10	Diabetes	Streptozotocin- and alloxan-induced or obesity-associated	Uchigata Y et al., Diabetes 1983, 32: 316-318. Masiello P et al., Diabetologia 1985, 28: 683-686. Shimabukuro M et al., J Clin Invest 1997, 100: 290-295.
15			
	Cancer	In vitro model; see below	Schlicker et al., 1999, 75(1), 91-100.

20

a) NMDA excitotoxicity model

Glutamate is the most important excitatory neurotransmitter in the brain. Under normal conditions, glutamate is secreted into the synaptic cleft and stimulates the post-synaptic glutamate receptors, specifically the glutamate receptors of the "NMDA" and "AMPA" types. This stimulation plays a significant part in numerous functions of the brain, including learning, memory and motor control.

30

Under the conditions of acute and chronic neurodegeneration (e.g. stroke), however, there is a great increase in the presynaptic glutamate secretion, resulting in excessive stimulation of the receptors. This leads to death of the cells stimulated in this way. These increased glutamate activities occur in a number of neurological disorders or psychological disturbances and lead to states of overexcitation or toxic effects in the central nervous system (CNS) but also in the peripheral nervous system. Thus, glutamate is involved in a large number of neurodegenerative disorders, in particular neurotoxic disturbances following hypoxia, anoxia, ischemia and after lesions like those occurring after stroke and trauma, and stroke, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS; "Lou Gehring's disease"), cranial trauma, spinal cord trauma, peripheral neuropathies, AIDS dementia and Parkinson's disease. Another disease in which glutamate receptors are important is epilepsy (cf. Brain

43

Res Bull 1998; 46(4):281-309, Eur Neuropsychopharmacol 1998, 8(2):141-52.).

Glutamate effects are mediated through various receptors. One of
5 these receptors is called the NMDA (N-methyl-D-aspartate) recep-
tor after a specific agonist (Arzneim.Forschung 1990, 40,
511-514; TIPS, 1990, 11, 334-338; Drugs of the Future 1989, 14,
1059-1071). N-Methyl-D-aspartate is a strong agonist of a par-
ticular class of glutamate receptors ("NMDA" type). Stimulation
10 of the NMDA receptor leads to influx of calcium into the cell and
the generation of free radicals. The free radicals lead to DNA
damage and activation of PARP. PARP in turn causes cell death
through depletion of high-energy phosphates (NAD and ATP) in the
cell. This explains the toxicity of NMDA. Treatment of animals
15 with NMDA can therefore be regarded as a model of the abovementioned disorders in which excitotoxicity is involved.

Because of the importance of glutamate receptors in neurodegener-
ation, many pharmacological approaches to date have been directed
20 at specific blocking of precisely these receptors. However, be-
cause of their importance in normal stimulus conduction, these
approaches have proved to be problematic (side effects). In addi-
tion, stimulation of the receptors is an event which takes place
very rapidly so that administration of the receptors often comes
25 too late ("time window" problem). Thus there is a great need for
novel principles of action and inhibitors of NMDA-related neuro-
toxicity.

Protection against cerebral overexcitation by excitatory amino
30 acids (NMDA antagonism in mice) can be regarded as adequate proof
of the activity of a pharmacological effector of PARP in dis-
orders based on excitotoxicity. Intracerebral administration of
excitatory amino acids (EAA) induces such massive overexcitation
that it leads within a short time to convulsions and death of the
35 animals (mice).

In the present case there was unilateral intracerebroventricular
administration of 10 µl of a 0.035% strength aqueous NMDA solution
120 minutes after intraperitoneal (i.p.) administration of the
40 test substance. These symptoms can be inhibited by systemic, e.g.
intraperitoneal, administration of centrally acting drugs. Since
excessive activation of EAA receptors in the central nervous
system plays an important part in the pathogenesis of various
neurological disorders, information can be gained from the
45 detected EAA antagonism in vivo about possible therapeutic
utilizability of the substances for such CNS disorders. An ED50
at which 50% of the animals are, due to preceding i.p.

44

administration of the measured substance, free of symptoms with a fixed dose of NMDA was determined as a measure of the activity of the substances.

5 b) Langendorf heart model (model for myocardial infarct)

Male Sprague-Dawley rats (bodyweight 300-400 g; origin Janvier, Le Genest-St-Isle, France) were used for the test. The rats were treated orally by gavage with the active substance or placebo
10 (volume: 5 ml/kg). 50 minutes later, heparin is administered intraperitoneally (Liquemin N Roche, 125 IU/animal in 0.5 ml). The animals are anesthetized with Inactin® T133 (thiobetabarbitol sodium 10%), fixed on the operating table, tracheotomized and ventilated with a "Harvard ventilatory pump" (40 beats/min,
15 4.5 ml/beat). Thoracotomy was followed by immediate catheterization of the aorta, removal of the heart and immediate retrograde perfusion. The hearts were perfused with a constant pressure of 75 mmHg, which is achieved using a "Gilson Miniplus 2 perfusion pump". Composition of the perfusate (mmol/l): NaCl 118, KCl 4.7,
20 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 2.52, $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 1.64, NaHCO_3 24.88, KH_2PO_4 1.18, glucose 11. The temperature is kept at 37°C throughout the experiment. Functional parameters were continuously recorded using a "Gould 4-channel recorder". Measurements were made of the left-ventricular pressure (LVP; mmHg), LVEDP (mmHg), enzyme release
25 (creatine kinase, mU/ml/g), coronary flow rate (ml/min), HR (pulse rate, min^{-1}). The left-ventricular pressure was measured using a liquid-filled latex balloon and a Statham23 Db pressure transducer. The volume of the balloon was initially adjusted to reach an LVEDP (left-ventricular end-diastolic pressure) of about
30 12 mmHg. The $\text{dP/dt}_{\text{max}}$ (maximum pumping force) is derived from the pressure signal using a differentiator module. The heart rate was calculated from the pressure signal. The flow rate was determined using a drop counter (BMT Messtechnik GmbH Berlin). After an equilibration time of 20 minutes, the hearts were subjected to a
35 30-minute global ischemia by stopping the perfusate supply while keeping the temperature at 37°C. During the following 60-minute reperfusion period, samples of the perfusate were taken after 3, 5, 10, 15, 30, 45 and 60 min for analysis of creatine kinase (CK) activity. Means and standard deviations for the measured para-
40 meters were analyzed statistically (Dunnett test). The significance limit was $p=0.05$.

The experiment on rabbit hearts was carried out similarly. Male white New Zealand rabbits (obtained from: Interfauna) were used.
45 The hearts were prepared as described above for the rat model. The perfusion pressure was set at a maximum of 60 mmHg and the flow rate at about 25ml/min. The equilibration time was about

45

30 min. The substance was administered by infusion directly upstream of the heart. 15 min after starting the infusion, a 30-minute global ischemia was caused by stopping the flow while maintaining the temperature of the heart. A 30-minute reperfusion followed. Perfusate was taken for investigation of CK activity before administration of the substance, after 15 min and at various times (5, 10, 15, 20, 30 min) during the reperfusion. The following parameters were measured: LVP (mmHg), LVEDP, LVdP/dt, PP (mmHg), HR (pulse rate; beats/min), CK activity (U/min/g heart weight).

c) Animal model for acute kidney failure

The protective effect of intravenous administration of PARP inhibitors (4 days) on the kidney function of rats with postischemic acute kidney failure was investigated.

Male Sprague-Dawley rats (about 330 g at the start of the experiments; breeder: Charles River) were used. 10-15 animals were employed per experimental group. Administration of active substance/placebo took place continuously with an osmotic micropump into the femoral vein. Orbital blood was taken (1.5 ml of whole blood) under inhalation anesthesia with enflurane (Ethrane Abbot, Wiesbaden).

25

After the initial measurements (blood sample) and determination of the amount of urine excreted in 24h, the rats were anesthetized ("Nembutal", pentobarbital sodium, Sanofi CEVA; 50mg/kg i.p., volume injected 1.0 ml/kg) and fastened on a heatable operating table (37°C). 125 IU/kg heparin (Liquemin N, Roche) were administered i.v. into the caudal vein. The abdominal cavity was opened and the right kidney was exposed. The branching-off renal artery was exposed and clamped off superiorly using bulldog clamps (Diefenbach 38mm). The left renal artery was likewise exposed and clamped off (superiorly, about half way to the kidney). During the operation, an osmotic micropump was implanted into the femoral vein. The intestine was reinserted and the fluid loss was compensated with luke-warm 0.9% NaCl. The animals were covered with a moist cloth and kept warm under red light. After 40 min, the appearance of the kidneys was recorded, and the clamps were removed, first the right then the left. The intestine was put back and 2 drops of antibiotic (Tardomyocel, Bayer) were added. The abdominal wall was closed with sterile cat gut (Ethicon No.4) and treated once more with 1 drop of antibiotic. The epidermis was sutured with sterile Ethibond Exel (Ethicon) No.3/0, and the

46

suture was sprayed with Nebacetin N (Yamanouchi) wound spray. A tenth of a daily dose of drug/placebo is given as i.v. bolus.

Samples and blood were taken for investigating biochemical parameters in the serum and urine: Na, K, creatinine, protein (only in urine), on days 1, 2 and 4 of the experiment. In addition, the feed and water consumption, bodyweight and urine volume were recorded. After 14 days, the animals were sacrificed and the kidneys were assessed.

10

The assessment excluded all animals which died of an infarct during the experiment or showed an infarct at necropsy on day 14.

The creatinine clearance and the fractional sodium excretion were calculated as kidney function parameters, comparing treated animals with control and sham.

d) In vitro model for radiosensitization (tumor therapy)

MCF-7-cells (human breast carcinoma) were cultivated in Dulbecco's modified Eagle's medium with 10% heat-inactivated FCS and 2 mM L-glutamine. Cells were seeded out overnight in cell densities of 100, 1000 or 10,000 cells per well in a 6-well plate and then exposed to ionizing radiation with a dose in the range from 0 to 10 Gy (^{137}Cs , Shepard Mark, model I-68A, dose rate 3.28 Gy/min). 10 days after the irradiation, the experiment was assessed, counting colonies with fifty cells as positive.

e) Stroke model (focal cerebral ischemia; MCA (middle cerebral artery) occlusion on a rat)

30

A focal ischemia was performed by means of cauterisation of the right distal MCA on Sprague-Dawley or Long-Evans rats. The rats may be treated before or after the beginning of the MCA occlusion with modulators of the proteins of the invention. As a rule, doses of 1-10 mg/kg are chosen (bolus application), optionally followed by a continuous infusion of 0.5-5 mg/kg/h.

The rats are anesthetised with halothane in a mixture of 70 % nitrogen and 30 % oxygen (4% at initial phase and 0.8-1.2 % during the operation). The body temperature was permanently measured rectally and was kept constant at $37.5\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ by means of a controllable heating blanket. Moreover, arterial blood pressure, arterial pH, $\text{Pa}(\text{O}_2)$ and $\text{Pa}(\text{CO}_2)$ were optionally measured by means of a tail vein catheter. Thereafter, the focal ischemia was carried out using the method of Chen et al. (Stroke 17: 738-743; 1986) or Liu et al. (Am. J. Physiol. 256: H589-593; 1989) by means of continuous cauterisation of the distal part of the right

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MCA. When the operation was terminated, the animals were kept in a warm environment for a further 24 hours. Then they were killed with the use of CO₂ and decapitated. Their brains were taken, shock-frozen (dry ice or liquid nitrogen) and stored at -80 °C.

- 5 The brains were cut into 0.02 mm thick slices and every 20th cut was used for the subsequent analysis. The corresponding cuts are stained with cresyl violet (Nissl staining). Alternatively, TTC (2,3,4-triphenyltetrazoliumchloride) may be used for staining. The infarct volume may then be analysed under a microscope. For
10 exact quantification, a computer-based image analyzing software may be used (J. Cereb. Blood Flow Metabol. 10: 290-293; 1990).

f) Septic shock

- 15 Groups of 10 male C57/BL mice (body weight 18-20 g) are treated with LPS (lipopolysaccharide, from E. coli, LD₁₀₀ 20 mg/animal i. v.) plus galactosamine (20 mg/animal i. v.). the substance to be tested is applied i. p. or i. v. during three succeeding days (e. g. 1-10 mg/kg), with the first dose being administered 30
20 minutes after the LPS treatment. The death rate is determined every 12 hours. Alternatively, the substance may also be applied in several doses spread over the days.

g) Determination of altered gene expression in aging cells

25

The aging of cells is simulated by changing the cell culture media from the complete medium with a reduced serum concentration and thereafter is analysed by means of quantitative PCR or Northern Blotting (Linskens et al., Nucleic Acids Res. 1995, 23(16):

- 30 3244-51). As typical markers for the aging of the skin for example collagen or elastin may be used. Human fibroblasts or fibroblast cell lines are used which simulate the aging of the skin. Modulators of the proteins of the invention are added to the medium and their effect on the changing of the gene express-
35 ion is observed. An increased production of elastin in cells with a reduced aging process caused by means of said modulators may be observed.

40

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2330206.seq
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<140> 2.330.206

<141> 1999-06-04

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Leu Lys Val Gln Phe Asn Phe Leu Gln Leu Trp
      560                               565                               570

taaataaacc agagatctga tcttcaagca agaaaataag cagtgttgta cttgtgaatt 1785

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2330206.seq

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 20 25 30
 Ser Ser Pro Ala Lys Lys Thr Arg Arg Cys Gln Arg Gln Glu Ser Lys
 35 40 45
 Lys Met Pro Val Ala Gly Gly Lys Ala Asn Lys Asp Arg Thr Glu Asp
 50 55 60
 Lys Gln Asp Glu Ser Val Lys Ala Leu Leu Leu Lys Gly Lys Ala Pro
 65 70 75 80
 Val Asp Pro Glu Cys Thr Ala Lys Val Gly Lys Ala His Val Tyr Cys
 85 90 95
 Glu Gly Asn Asp Val Tyr Asp Val Met Leu Asn Gln Thr Asn Leu Gln
 100 105 110
 Phe Asn Asn Asn Lys Tyr Tyr Leu Ile Gln Leu Leu Glu Asp Asp Ala
 115 120 125
 Gln Arg Asn Phe Ser Val Trp Met Arg Trp Gly Arg Val Gly Lys Met
 130 135 140
 Gly Gln His Ser Leu Val Ala Cys Ser Gly Asn Leu Asn Lys Ala Lys
 145 150 155 160
 Glu Ile Phe Gln Lys Lys Phe Leu Asp Lys Thr Lys Asn Asn Trp Glu
 165 170 175
 Asp Arg Glu Lys Phe Glu Lys Val Pro Gly Lys Tyr Asp Met Leu Gln
 180 185 190
 Met Asp Tyr Ala Thr Asn Thr Gln Asp Glu Glu Glu Thr Lys Lys Glu
 195 200 205
 Glu Ser Leu Lys Ser Pro Leu Lys Pro Glu Ser Gln Leu Asp Leu Arg
 210 215 220
 Val Gln Glu Leu Ile Lys Leu Ile Cys Asn Val Gln Ala Met Glu Glu
 225 230 235 240
 Met Met Met Glu Met Lys Tyr Asn Thr Lys Lys Ala Pro Leu Gly Lys
 245 250 255
 Leu Thr Val Ala Gln Ile Lys Ala Gly Tyr Gln Ser Leu Lys Lys Ile
 260 265 270
 Glu Asp Cys Ile Arg Ala Gly Gln His Gly Arg Ala Leu Met Glu Ala
 275 280 285
 Cys Asn Glu Phe Tyr Thr Arg Ile Pro His Asp Phe Gly Leu Arg Thr
 290 295 300
 Pro Pro Leu Ile Arg Thr Gln Lys Glu Leu Ser Glu Lys Ile Gln Leu
 305 310 315 320
 Leu Glu Ala Leu Gly Asp Ile Glu Ile Ala Ile Lys Leu Val Lys Thr
 325 330 335
 Glu Leu Gln Ser Pro Glu His Pro Leu Asp Gln His Tyr Arg Asn Leu
 340 345 350

2330206.seq

His Cys Ala Leu Arg Pro Leu Asp His Glu Ser Tyr Glu Phe Lys Val
 355 360 365
 Ile Ser Gln Tyr Leu Gln Ser Thr His Ala Pro Thr His Ser Asp Tyr
 370 375 380
 Thr Met Thr Leu Leu Asp Leu Phe Glu Val Glu Lys Asp Gly Glu Lys
 385 390 395 400
 Glu Ala Phe Arg Glu Asp Leu His Asn Arg Met Leu Leu Trp His Gly
 405 410 415
 Ser Arg Met Ser Asn Trp Val Gly Ile Leu Ser His Gly Leu Arg Ile
 420 425 430
 Ala Pro Pro Glu Ala Pro Ile Thr Gly Tyr Met Phe Gly Lys Gly Ile
 435 440 445
 Tyr Phe Ala Asp Met Ser Ser Lys Ser Ala Asn Tyr Cys Phe Ala Ser
 450 455 460
 Arg Leu Lys Asn Thr Gly Leu Leu Leu Leu Ser Glu Val Ala Leu Gly
 465 470 475 480
 Gln Cys Asn Glu Leu Leu Glu Ala Asn Pro Lys Ala Glu Gly Leu Leu
 485 490 495
 Gln Gly Lys His Ser Thr Lys Gly Leu Gly Lys Met Ala Pro Ser Ser
 500 505 510
 Ala His Phe Val Thr Leu Asn Gly Ser Thr Val Pro Leu Gly Pro Ala
 515 520 525
 Ser Asp Thr Gly Ile Leu Asn Pro Asp Gly Tyr Thr Leu Asn Tyr Asn
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<222> (242)..(1843)

<223> Product=Poly ADP Ribose Polymerase

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 tctccctaatt tcacgcctga ggctcatgga gagttgctag acctgggact gccctgggag 180
 gcgcacacaa ccaggccggg tggcagccag gacctctccc atgtccctgc ttttcttggc 240
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2330206.seq

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Lys	Lys	Gly	Arg	Gln	Ala	Gly	Arg	Glu	Glu	Asp	Pro	Phe	Arg	Ser	Thr	
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gct	gag	gcc	ctc	aag	gcc	ata	ccc	gca	gag	aag	cgc	ata	atc	cgc	gtg	385
Ala	Glu	Ala	Leu	Lys	Ala	Ile	Pro	Ala	Glu	Lys	Arg	Ile	Ile	Arg	Val	
		35					40					45				
gat	cca	aca	tgt	cca	ctc	agc	agc	aac	ccc	ggg	acc	cag	gtg	tat	gag	433
Asp	Pro	Thr	Cys	Pro	Leu	Ser	Ser	Asn	Pro	Gly	Thr	Gln	Val	Tyr	Glu	
	50					55					60					
gac	tac	aac	tgc	acc	ctg	aac	cag	acc	aac	atc	gag	aac	aac	aac	aac	481
Asp	Tyr	Asn	Cys	Thr	Leu	Asn	Gln	Thr	Asn	Ile	Glu	Asn	Asn	Asn	Asn	
	65				70				75						80	
aag	ttc	tac	atc	atc	cag	ctg	ctc	caa	gac	agc	aac	cgc	ttc	ttc	acc	529
Lys	Phe	Tyr	Ile	Ile	Gln	Leu	Leu	Gln	Asp	Ser	Asn	Arg	Phe	Phe	Thr	
			85					90						95		
tgc	tgg	aac	cgc	tgg	ggc	cgt	gtg	gga	gag	gtc	ggc	cag	tca	aag	atc	577
Cys	Trp	Asn	Arg	Trp	Gly	Arg	Val	Gly	Glu	Val	Gly	Gln	Ser	Lys	Ile	
			100					105				110				
aac	cac	ttc	aca	agg	cta	gaa	gat	gca	aag	aag	gac	ttt	gag	aag	aaa	625
Asn	His	Phe	Thr	Arg	Leu	Glu	Asp	Ala	Lys	Lys	Asp	Phe	Glu	Lys	Lys	
		115					120					125				
ttt	cgg	gaa	aag	acc	aag	aac	aac	tgg	gca	gag	cgg	gac	cac	ttt	gtg	673
Phe	Arg	Glu	Lys	Thr	Lys	Asn	Asn	Trp	Ala	Glu	Arg	Asp	His	Phe	Val	
	130					135					140					
tct	cac	ccg	ggc	aag	tac	aca	ctt	atc	gaa	gta	cag	gca	gag	gat	gag	721
Ser	His	Pro	Gly	Lys	Tyr	Thr	Leu	Ile	Glu	Val	Gln	Ala	Glu	Asp	Glu	
	145				150				155						160	
gcc	cag	gaa	gct	gtg	gtg	aag	gtg	gac	aga	ggc	cca	gtg	agg	act	gtg	769
Ala	Gln	Glu	Ala	Val	Val	Lys	Val	Asp	Arg	Gly	Pro	Val	Arg	Thr	Val	
			165					170						175		
act	aag	cgg	gtg	cag	ccc	tgc	tcc	ctg	gac	cca	gcc	acg	cag	aag	ctc	817
Thr	Lys	Arg	Val	Gln	Pro	Cys	Ser	Leu	Asp	Pro	Ala	Thr	Gln	Lys	Leu	
			180					185					190			
atc	act	aac	atc	ttc	agc	aag	gag	atg	ttc	aag	aac	acc	atg	gcc	ctc	865
Ile	Thr	Asn	Ile	Phe	Ser	Lys	Glu	Met	Phe	Lys	Asn	Thr	Met	Ala	Leu	
		195					200					205				
atg	gac	ctg	gat	gtg	aag	aag	atg	ccc	ctg	gga	aag	ctg	agc	aag	caa	913
Met	Asp	Leu	Asp	Val	Lys	Lys	Met	Pro	Leu	Gly	Lys	Leu	Ser	Lys	Gln	
	210					215					220					
cag	att	gca	cgg	ggt	ttc	gag	gcc	ttg	gag	gcg	ctg	gag	gag	gcc	ctg	961
Gln	Ile	Ala	Arg	Gly	Phe	Glu	Ala	Leu	Glu	Ala	Leu	Glu	Glu	Ala	Leu	
	225				230					235					240	
aaa	ggc	ccc	acg	gat	ggt	ggc	caa	agc	ctg	gag	gag	ctg	tcc	tca	cac	1009
Lys	Gly	Pro	Thr	Asp	Gly	Gly	Gln	Ser	Leu	Glu	Glu	Leu	Ser	Ser	His	
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ttt	tac	acc	gtc	atc	ccg	cac	aac	ttc	ggc	cac	agc	cag	ccc	ccg	ccc	1057

2330206.seq

Phe	Tyr	Thr	Val	Ile	Pro	His	Asn	Phe	Gly	His	Ser	Gln	Pro	Pro	Pro	
			260					265					270			
atc	aat	tcc	cct	gag	ctt	ctg	cag	gcc	aag	aag	gac	atg	ctg	ctg	gtg	1105
Ile	Asn	Ser	Pro	Glu	Leu	Leu	Gln	Ala	Lys	Lys	Asp	Met	Leu	Leu	Val	
		275					280					285				
ctg	gcg	gac	atc	gag	ctg	gcc	cag	gcc	ctg	cag	gca	gtc	tct	gag	cag	1153
Leu	Ala	Asp	Ile	Glu	Leu	Ala	Gln	Ala	Leu	Gln	Ala	Val	Ser	Glu	Gln	
		290				295					300					
gag	aag	acg	gtg	gag	gag	gtg	cca	cac	ccc	ctg	gac	cga	gac	tac	cag	1201
Glu	Lys	Thr	Val	Glu	Glu	Val	Pro	His	Pro	Leu	Asp	Arg	Asp	Tyr	Gln	
305					310					315					320	
ctt	ctc	aag	tgc	cag	ctg	cag	ctg	cta	gac	tct	gga	gca	cct	gag	tac	1249
Leu	Leu	Lys	Cys	Gln	Leu	Gln	Leu	Leu	Asp	Ser	Gly	Ala	Pro	Glu	Tyr	
				325					330					335		
aag	gtg	ata	cag	acc	tac	tta	gaa	cag	act	ggc	agc	aac	cac	agg	tgc	1297
Lys	Val	Ile	Gln	Thr	Tyr	Leu	Glu	Gln	Thr	Gly	Ser	Asn	His	Arg	Cys	
			340					345					350			
cct	aca	ctt	caa	cac	atc	tgg	aaa	gta	aac	caa	gaa	ggg	gag	gaa	gac	1345
Pro	Thr	Leu	Gln	His	Ile	Trp	Lys	Val	Asn	Gln	Glu	Gly	Glu	Glu	Asp	
		355					360					365				
aga	ttc	cag	gcc	cac	tcc	aaa	ctg	ggt	aat	cgg	aag	ctg	ctg	tgg	cat	1393
Arg	Phe	Gln	Ala	His	Ser	Lys	Leu	Gly	Asn	Arg	Lys	Leu	Leu	Trp	His	
		370				375					380					
ggc	acc	aac	atg	gcc	gtg	gtg	gcc	gcc	atc	ctc	act	agt	ggg	ctc	cgc	1441
Gly	Thr	Asn	Met	Ala	Val	Val	Ala	Ala	Ile	Leu	Thr	Ser	Gly	Leu	Arg	
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atc	atg	cca	cat	tct	ggt	ggg	cgt	ggt	ggc	aag	ggc	atc	tac	ttt	gcc	1489
Ile	Met	Pro	His	Ser	Gly	Gly	Arg	Val	Gly	Lys	Gly	Ile	Tyr	Phe	Ala	
				405					410					415		
tca	gag	aac	agc	aag	tca	gct	gga	tat	ggt	att	ggc	atg	aag	tgt	ggg	1537
Ser	Glu	Asn	Ser	Lys	Ser	Ala	Gly	Tyr	Val	Ile	Gly	Met	Lys	Cys	Gly	
			420					425					430			
gcc	cac	cat	gtc	ggc	tac	atg	ttc	ctg	ggt	gag	gtg	gcc	ctg	ggc	aga	1585
Ala	His	His	Val	Gly	Tyr	Met	Phe	Leu	Gly	Glu	Val	Ala	Leu	Gly	Arg	
		435					440					445				
gag	cac	cat	atc	aac	acg	gac	aac	ccc	agc	ttg	aag	agc	cca	cct	cct	1633
Glu	His	His	Ile	Asn	Thr	Asp	Asn	Pro	Ser	Leu	Lys	Ser	Pro	Pro	Pro	
		450				455					460					
ggc	ttc	gac	agt	gtc	att	gcc	cga	ggc	cac	acc	gag	cct	gat	ccg	acc	1681
Gly	Phe	Asp	Ser	Val	Ile	Ala	Arg	Gly	His	Thr	Glu	Pro	Asp	Pro	Thr	
465					470					475					480	
cag	gac	act	gag	ttg	gag	ctg	gat	ggc	cag	caa	gtg	gtg	gtg	ccc	cag	1729
Gln	Asp	Thr	Glu	Leu	Glu	Leu	Asp	Gly	Gln	Gln	Val	Val	Val	Pro	Gln	
				485					490					495		
ggc	cag	cct	gtg	ccc	tgc	cca	gag	ttc	agc	agc	tcc	aca	ttc	tcc	cag	1777
Gly	Gln	Pro	Val	Pro	Cys	Pro	Glu	Phe	Ser	Ser	Ser	Thr	Phe	Ser	Gln	
			500					505					510			
agc	gag	tac	ctc	atc	tac	cag	gag	agc	cag	tgt	cgc	ctg	cgc	tac	ctg	1825

2330206.seq

Ser Glu Tyr Leu Ile Tyr Gln Glu Ser Gln Cys Arg Leu Arg Tyr Leu
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ctg gag gtc cac ctc tga gtgccccgcc tgccccccgg ggtcctgcaa 1873
 Leu Glu Val His Leu
 530

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 tttcaagaat acaatacgtt gttgttaact atagtcacca tgctgtacaa gatccctgaa 1993
 cttatgcctc ctaactgaaa ttttgtattc ttgacacat ctgcccagtc cctctcctcc 2053
 cagcccatgg taaccagcat ttgactcttt acttgtataa gggcagcttt tataggttcc 2113
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 aatgtgcacc gggttcaccc atgttttcat aaatgacaag atttctcctc ttaaaaaaaaaa 2233
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 2265

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 35 40 45

Asp Pro Thr Cys Pro Leu Ser Ser Asn Pro Gly Thr Gln Val Tyr Glu
 50 55 60

Asp Tyr Asn Cys Thr Leu Asn Gln Thr Asn Ile Glu Asn Asn Asn Asn
 65 70 75 80

Lys Phe Tyr Ile Ile Gln Leu Leu Gln Asp Ser Asn Arg Phe Phe Thr
 85 90 95

Cys Trp Asn Arg Trp Gly Arg Val Gly Glu Val Gly Gln Ser Lys Ile
 100 105 110

Asn His Phe Thr Arg Leu Glu Asp Ala Lys Lys Asp Phe Glu Lys Lys
 115 120 125

Phe Arg Glu Lys Thr Lys Asn Asn Trp Ala Glu Arg Asp His Phe Val
 130 135 140

Ser His Pro Gly Lys Tyr Thr Leu Ile Glu Val Gln Ala Glu Asp Glu
 145 150 155 160

Ala Gln Glu Ala Val Val Lys Val Asp Arg Gly Pro Val Arg Thr Val
 165 170 175

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

2330206.seq

Ile Thr Asn Ile Phe Ser Lys Glu Met Phe Lys Asn Thr Met Ala Leu
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 Met Asp Leu Asp Val Lys Lys Met Pro Leu Gly Lys Leu Ser Lys Gln
 210 215 220
 Gln Ile Ala Arg Gly Phe Glu Ala Leu Glu Ala Leu Glu Glu Ala Leu
 225 230 235 240
 Lys Gly Pro Thr Asp Gly Gly Gln Ser Leu Glu Glu Leu Ser Ser His
 245 250 255
 Phe Tyr Thr Val Ile Pro His Asn Phe Gly His Ser Gln Pro Pro Pro
 260 265 270
 Ile Asn Ser Pro Glu Leu Leu Gln Ala Lys Lys Asp Met Leu Leu Val
 275 280 285
 Leu Ala Asp Ile Glu Leu Ala Gln Ala Leu Gln Ala Val Ser Glu Gln
 290 295 300
 Glu Lys Thr Val Glu Glu Val Pro His Pro Leu Asp Arg Asp Tyr Gln
 305 310 315 320
 Leu Leu Lys Cys Gln Leu Gln Leu Leu Asp Ser Gly Ala Pro Glu Tyr
 325 330 335
 Lys Val Ile Gln Thr Tyr Leu Glu Gln Thr Gly Ser Asn His Arg Cys
 340 345 350
 Pro Thr Leu Gln His Ile Trp Lys Val Asn Gln Glu Gly Glu Glu Asp
 355 360 365
 Arg Phe Gln Ala His Ser Lys Leu Gly Asn Arg Lys Leu Leu Trp His
 370 375 380
 Gly Thr Asn Met Ala Val Val Ala Ala Ile Leu Thr Ser Gly Leu Arg
 385 390 395 400
 Ile Met Pro His Ser Gly Gly Arg Val Gly Lys Gly Ile Tyr Phe Ala
 405 410 415
 Ser Glu Asn Ser Lys Ser Ala Gly Tyr Val Ile Gly Met Lys Cys Gly
 420 425 430
 Ala His His Val Gly Tyr Met Phe Leu Gly Glu Val Ala Leu Gly Arg
 435 440 445
 Glu His His Ile Asn Thr Asp Asn Pro Ser Leu Lys Ser Pro Pro Pro
 450 455 460
 Gly Phe Asp Ser Val Ile Ala Arg Gly His Thr Glu Pro Asp Pro Thr
 465 470 475 480
 Gln Asp Thr Glu Leu Glu Leu Asp Gly Gln Gln Val Val Val Pro Gln
 485 490 495
 Gly Gln Pro Val Pro Cys Pro Glu Phe Ser Ser Ser Thr Phe Ser Gln
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<223> Product=Poly ADP Ribose Polymerase
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tctccctaat tcacgectga ggctcatgga gagttgctag acctgggact gccctgggag 180
gcgcacacaa ccaggccggg tggcagccag gacctctccc atg tcc ctg ctt ttc 235
                Met Ser Leu Leu Phe
                1       5

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ttg gcc atg gct cca aag ccg aag ccc tgg gta cag act gag ggc cct 283
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gag aag aag aag ggc cgg cag gca gga agg gag gag gac ccc ttc cgc 331
Glu Lys Lys Lys Gly Arg Gln Ala Gly Arg Glu Glu Asp Pro Phe Arg
25 30 35

tcc acc gct gag gcc ctc aag gcc ata ccc gca gag aag cgc ata atc 379
Ser Thr Ala Glu Ala Leu Lys Ala Ile Pro Ala Glu Lys Arg Ile Ile
40 45 50

cgc gtg gat cca aca tgt cca ctc agc agc aac ccc ggg acc cag gtg 427
Arg Val Asp Pro Thr Cys Pro Leu Ser Ser Asn Pro Gly Thr Gln Val
55 60 65

tat	gag	gac	tac	aac	tgc	acc	ctg	aac	cag	acc	aac	atc	gag	aac	aac	475
Tyr	Glu	Asp	Tyr	Asn	Cys	Thr	Leu	Asn	Gln	Thr	Asn	Ile	Glu	Asn	Asn	
70					75					80					85	

aac aac aag ttc tac atc atc cag ctg ctc caa gac agc aac cgc ttc 523
Asn Asn Lys Phe Tyr Ile Ile Gln Leu Leu Gln Asp Ser Asn Arg Phe
90 95 100

ttc	acc	tgc	tgg	aac	cgc	tgg	ggc	cgt	gtg	gga	gag	gtc	ggc	cag	tca	571
Phe	Thr	Cys	Trp	Asn	Arg	Trp	Gly	Arg	Val	Gly	Glu	Val	Gly	Gln	Ser	
			105					110					115			

aag atc aac cac ttc aca agg cta gaa gat gca aag aag gac ttt gag 619
Lys Ile Asn His Phe Thr Arg Leu Glu Asp Ala Lys Lys Asp Phe Glu
120 125 130

aag aaa ttt cgg gaa aag acc aag aac aac tgg gca gag cgg gac cac 667
Lys Lys Phe Arg Glu Lys Thr Lys Asn Asn Trp Ala Glu Arg Asp His
135 140 145

[illegible]

2330206.seq

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Thr Val Thr Lys Arg Val Gln Pro Cys Ser Leu Asp Pro Ala Thr Gln	
185 190 195	
aag ctc atc act aac atc ttc agc aag gag atg ttc aag aac acc atg	859
Lys Leu Ile Thr Asn Ile Phe Ser Lys Glu Met Phe Lys Asn Thr Met	
200 205 210	
gcc ctc atg gac ctg gat gtg aag aag atg ccc ctg gga aag ctg agc	907
Ala Leu Met Asp Leu Asp Val Lys Lys Met Pro Leu Gly Lys Leu Ser	
215 220 225	
aag caa cag att gca cgg ggt ttc gag gcc ttg gag gcg ctg gag gag	955
Lys Gln Gln Ile Ala Arg Gly Phe Glu Ala Leu Glu Ala Leu Glu Glu	
230 235 240 245	
gcc ctg aaa ggc ccc acg gat ggt ggc caa agc ctg gag gag ctg tcc	1003
Ala Leu Lys Gly Pro Thr Asp Gly Gly Gln Ser Leu Glu Glu Leu Ser	
250 255 260	
tca cac ttt tac acc gtc atc ccg cac aac ttc ggc cac agc cag ccc	1051
Ser His Phe Tyr Thr Val Ile Pro His Asn Phe Gly His Ser Gln Pro	
265 270 275	
ccg ccc atc aat tcc cct gag ctt ctg cag gcc aag aag gac atg ctg	1099
Pro Pro Ile Asn Ser Pro Glu Leu Leu Gln Ala Lys Lys Asp Met Leu	
280 285 290	
ctg gtg ctg gcg gac atc gag ctg gcc cag gcc ctg cag gca gtc tct	1147
Leu Val Leu Ala Asp Ile Glu Leu Ala Gln Ala Leu Gln Ala Val Ser	
295 300 305	
gag cag gag aag acg gtg gag gag gtg cca cac ccc ctg gac cga gac	1195
Glu Gln Glu Lys Thr Val Glu Glu Val Pro His Pro Leu Asp Arg Asp	
310 315 320 325	
tac cag ctt ctc aag tgc cag ctg cag ctg cta gac tct gga gca cct	1243
Tyr Gln Leu Leu Lys Cys Gln Leu Gln Leu Leu Asp Ser Gly Ala Pro	
330 335 340	
gag tac aag gtg ata cag acc tac tta gaa cag act ggc agc aac cac	1291
Glu Tyr Lys Val Ile Gln Thr Tyr Leu Glu Gln Thr Gly Ser Asn His	
345 350 355	
agg tgc cct aca ctt caa cac atc tgg aaa gta aac caa gaa ggg gag	1339
Arg Cys Pro Thr Leu Gln His Ile Trp Lys Val Asn Gln Glu Gly Glu	
360 365 370	
gaa gac aga ttc cag gcc cac tcc aaa ctg ggt aat cgg aag ctg ctg	1387
Glu Asp Arg Phe Gln Ala His Ser Lys Leu Gly Asn Arg Lys Leu Leu	
375 380 385	
tgg cat ggc acc aac atg gcc gtg gtg gcc gcc atc ctc act agt ggg	1435
Trp His Gly Thr Asn Met Ala Val Val Ala Ala Ile Leu Thr Ser Gly	
390 395 400 405	
ctc cgc atc atg cca cat tct ggt ggg cgt gtt ggc aag ggc atc tac	1483
Leu Arg Ile Met Pro His Ser Gly Gly Arg Val Gly Lys Gly Ile Tyr	
410 415 420	

2330206.seq

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Phe Ala Ser Glu Asn Ser Lys Ser Ala Gly Tyr Val Ile Gly Met Lys
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tgt ggg gcc cac cat gtc ggc tac atg ttc ctg ggt gag gtg gcc ctg 1579
Cys Gly Ala His His Val Gly Tyr Met Phe Leu Gly Glu Val Ala Leu
               440                      445                      450

ggc aga gag cac cat atc aac acg gac aac ccc agc ttg aag agc cca 1627
Gly Arg Glu His His Ile Asn Thr Asp Asn Pro Ser Leu Lys Ser Pro
               455                      460                      465

cct cct ggc ttc gac agt gtc att gcc cga ggc cac acc gag cct gat 1675
Pro Pro Gly Phe Asp Ser Val Ile Ala Arg Gly His Thr Glu Pro Asp
               470                      475                      480                      485

ccg acc cag gac act gag ttg gag ctg gat ggc cag caa gtg gtg gtg 1723
Pro Thr Gln Asp Thr Glu Leu Glu Leu Asp Gly Gln Gln Val Val Val
               490                      495                      500

ccc cag ggc cag cct gtg ccc tgc cca gag ttc agc agc tcc aca ttc 1771
Pro Gln Gly Gln Pro Val Pro Cys Pro Glu Phe Ser Ser Ser Thr Phe
               505                      510                      515

tcc cag agc gag tac ctc atc tac cag gag agc cag tgt cgc ctg cgc 1819
Ser Gln Ser Glu Tyr Leu Ile Tyr Gln Glu Ser Gln Cys Arg Leu Arg
               520                      525                      530

tac ctg ctg gag gtc cac ctc tga gtgccccgcc tgtcccccggt ggtcctgcaa 1873
Tyr Leu Leu Glu Val His Leu
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ggctggactg tgatcttcaa tcacctgcc catctctggt acccctatat cactcctttt 1933

tttcaagaat acaatacggt gttgttaact atagtcacca tgctgtacaa gatccctgaa 1993

cttatgcctc ctaactgaaa ttttgtattc ttgacacat ctgccagtc cctctcctcc 2053

cagcccatgg taaccagcat ttgactcttt acttgataaa gggcagcttt tatagggttcc 2113

acatgtaagt gagatcatgc agtgtttgtc tttctgtgcc tggcttattt cactcagcat 2173

aatgtgcacc ggggttcaccc atgttttcat aaatgacaag atttcctcct ttaaaaaaaaaa 2233

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Glu Asp Pro Phe Arg Ser Thr Ala Glu Ala Leu Lys Ala Ile Pro Ala
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Glu Lys Arg Ile Ile Arg Val Asp Pro Thr Cys Pro Leu Ser Ser Asn

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2330206.seq
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 Asn Ile Glu Asn Asn Asn Asn Lys Phe Tyr Ile Ile Gln Leu Leu Gln
 85 90 95
 Asp Ser Asn Arg Phe Phe Thr Cys Trp Asn Arg Trp Gly Arg Val Gly
 100 105 110
 Glu Val Gly Gln Ser Lys Ile Asn His Phe Thr Arg Leu Glu Asp Ala
 115 120 125
 Lys Lys Asp Phe Glu Lys Lys Phe Arg Glu Lys Thr Lys Asn Asn Trp
 130 135 140
 Ala Glu Arg Asp His Phe Val Ser His Pro Gly Lys Tyr Thr Leu Ile
 145 150 155 160
 Glu Val Gln Ala Glu Asp Glu Ala Gln Glu Ala Val Val Lys Val Asp
 165 170 175
 Arg Gly Pro Val Arg Thr Val Thr Lys Arg Val Gln Pro Cys Ser Leu
 180 185 190
 Asp Pro Ala Thr Gln Lys Leu Ile Thr Asn Ile Phe Ser Lys Glu Met
 195 200 205
 Phe Lys Asn Thr Met Ala Leu Met Asp Leu Asp Val Lys Lys Met Pro
 210 215 220
 Leu Gly Lys Leu Ser Lys Gln Gln Ile Ala Arg Gly Phe Glu Ala Leu
 225 230 235 240
 Glu Ala Leu Glu Glu Ala Leu Lys Gly Pro Thr Asp Gly Gly Gln Ser
 245 250 255
 Leu Glu Glu Leu Ser Ser His Phe Tyr Thr Val Ile Pro His Asn Phe
 260 265 270
 Gly His Ser Gln Pro Pro Pro Ile Asn Ser Pro Glu Leu Leu Gln Ala
 275 280 285
 Lys Lys Asp Met Leu Leu Val Leu Ala Asp Ile Glu Leu Ala Gln Ala
 290 295 300
 Leu Gln Ala Val Ser Glu Gln Glu Lys Thr Val Glu Glu Val Pro His
 305 310 315 320
 Pro Leu Asp Arg Asp Tyr Gln Leu Leu Lys Cys Gln Leu Gln Leu Leu
 325 330 335
 Asp Ser Gly Ala Pro Glu Tyr Lys Val Ile Gln Thr Tyr Leu Glu Gln
 340 345 350
 Thr Gly Ser Asn His Arg Cys Pro Thr Leu Gln His Ile Trp Lys Val
 355 360 365
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 Asn Arg Lys Leu Leu Trp His Gly Thr Asn Met Ala Val Val Ala Ala
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                                     Met Ala
                                     1
cca aaa cga aag gcc tct gtg cag act gag ggc tcc aag aag cag cga 165
Pro Lys Arg Lys Ala Ser Val Gln Thr Glu Gly Ser Lys Lys Gln Arg
          5                      10                      15
caa ggg aca gag gag gag gac agc ttc cgg tcc act gcc gag gct ctc 213
Gln Gly Thr Glu Glu Glu Asp Ser Phe Arg Ser Thr Ala Glu Ala Leu
          20                      25                      30
aga gca gca cct gct gat aat cgg gtc atc cgt gtg gac ccc tca tgt 261
Arg Ala Ala Pro Ala Asp Asn Arg Val Ile Arg Val Asp Pro Ser Cys
          35                      40                      45                      50
cca ttc agc cgg aac ccc ggg ata cag gtc cac gag gac tat gac tgt 309
Pro Phe Ser Arg Asn Pro Gly Ile Gln Val His Glu Asp Tyr Asp Cys
          55                      60                      65
acc ctg aac cag acc aac atc ggc aac aac aac aac aag ttc tat att 357
Thr Leu Asn Gln Thr Asn Ile Gly Asn Asn Asn Asn Asn Lys Phe Tyr Ile

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85	90	95	
ggc cgc gtg gga gag gtg ggc cag agc aag atg aac cac ttc acc tgc			453
Gly Arg Val Gly Glu Val Gly Gln Ser Lys Met Asn His Phe Thr Cys			
100	105	110	
ctg gaa gat gca aag aag gac ttt aag aag aaa ttt tgg gag aag act			501
Leu Glu Asp Ala Lys Lys Asp Phe Lys Lys Lys Phe Trp Glu Lys Thr			
115	120	125	130
aaa aac aaa tgg gag gag cgg gac cgt ttt gtg gcc cag ccc aac aag			549
Lys Asn Lys Trp Glu Glu Arg Asp Arg Phe Val Ala Gln Pro Asn Lys			
135	140	145	
tac aca ctt ata gaa gtc cag gga gaa gca gag agc caa gag gct gta			597
Tyr Thr Leu Ile Glu Val Gln Gly Glu Ala Glu Ser Gln Glu Ala Val			
150	155	160	
gtg aag gcc tta tct ccc cag gtg gac agc ggc cct gtg agg acc gtg			645
Val Lys Ala Leu Ser Pro Gln Val Asp Ser Gly Pro Val Arg Thr Val			
165	170	175	
gtc aag ccc tgc tcc cta gac cct gcc acc cag aac ctt atc acc aac			693
Val Lys Pro Cys Ser Leu Asp Pro Ala Thr Gln Asn Leu Ile Thr Asn			
180	185	190	
atc ttc agc aaa gag atg ttc aag aac gca atg acc ctc atg aac ctg			741
Ile Phe Ser Lys Glu Met Phe Lys Asn Ala Met Thr Leu Met Asn Leu			
195	200	205	210
gat gtg aag aag atg ccc ttg gga aag ctg acc aag cag cag att gcc			789
Asp Val Lys Lys Met Pro Leu Gly Lys Leu Thr Lys Gln Gln Ile Ala			
215	220	225	
cgt ggc ttc gag gcc ttg gaa gct cta gag gag gcc atg aaa aac ccc			837
Arg Gly Phe Glu Ala Leu Glu Ala Leu Glu Glu Ala Met Lys Asn Pro			
230	235	240	
aca ggg gat ggc cag agc ctg gaa gag ctc tcc tcc tgc ttc tac act			885
Thr Gly Asp Gly Gln Ser Leu Glu Glu Leu Ser Ser Cys Phe Tyr Thr			
245	250	255	
gtc atc cca cac aac ttc ggc cgc agc cga ccc ccg ccc atc aac tcc			933
Val Ile Pro His Asn Phe Gly Arg Ser Arg Pro Pro Pro Ile Asn Ser			
260	265	270	
cct gat gtg ctt cag gcc aag aag gac atg ctg ctg gtg cta gcg gac			981
Pro Asp Val Leu Gln Ala Lys Lys Asp Met Leu Leu Val Leu Ala Asp			
275	280	285	290
atc gag ttg gcg cag acc ttg cag gca gcc cct ggg gag gag gag gag			1029
Ile Glu Leu Ala Gln Thr Leu Gln Ala Ala Pro Gly Glu Glu Glu Glu			
295	300	305	
aaa gtg gaa gag gtg cca cac cca ctg gat cga gac tac cag ctc ctc			1077
Lys Val Glu Glu Val Pro His Pro Leu Asp Arg Asp Tyr Gln Leu Leu			
310	315	320	
agg tgc cag ctt caa ctg ctg gac tcc ggg gag tcc gag tac aag gca			1125
Arg Cys Gln Leu Gln Leu Leu Asp Ser Gly Glu Ser Glu Tyr Lys Ala			

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325          330          335
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340          345          350

ctg cgg cat gtt tgg aaa gtg aac cga gaa ggg gag gga gac agg ttc 1221
Leu Arg His Val Trp Lys Val Asn Arg Glu Gly Glu Gly Asp Arg Phe
355          360          365

cag gcc cac tcc aaa ctg ggc aat cgg agg ctg ctg tgg cac ggc acc 1269
Gln Ala His Ser Lys Leu Gly Asn Arg Arg Leu Leu Trp His Gly Thr
375          380          385

aat gtg gcc gtg gtg gct gcc atc ctc acc agt ggg ctc cga atc atg 1317
Asn Val Ala Val Val Ala Ala Ile Leu Thr Ser Gly Leu Arg Ile Met
390          395          400

cca cac tcg ggt ggt cgt gtt ggc aag ggt att tat ttt gcc tct gag 1365
Pro His Ser Gly Gly Arg Val Gly Lys Gly Ile Tyr Phe Ala Ser Glu
405          410          415

aac agc aag tca gct ggc tat gtt acc acc atg cac tgt ggg ggc cac 1413
Asn Ser Lys Ser Ala Gly Tyr Val Thr Thr Met His Cys Gly Gly His
420          425          430

cag gtg ggc tac atg ttc ctg ggc gag gtg gcc ctc ggc aaa gag cac 1461
Gln Val Gly Tyr Met Phe Leu Gly Glu Val Ala Leu Gly Lys Glu His
435          440          445

cac atc acc atc gat gac ccc agc ttg aag agt cca ccc cct ggc ttt 1509
His Ile Thr Ile Asp Asp Pro Ser Leu Lys Ser Pro Pro Pro Gly Phe
455          460          465

gac agc gtc atc gcc cga ggc caa acc gag ccg gat ccc gcc cag gac 1557
Asp Ser Val Ile Ala Arg Gly Gln Thr Glu Pro Asp Pro Ala Gln Asp
470          475          480

att gaa ctt gaa ctg gat ggg cag ccg gtg gtg gtg ccc caa ggc ccg 1605
Ile Glu Leu Glu Leu Asp Gly Gln Pro Val Val Val Pro Gln Gly Pro
485          490          495

cct gtg cag tgc ccg tca ttc aaa agc tcc agc ttc agc cag agt gaa 1653
Pro Val Gln Cys Pro Ser Phe Lys Ser Ser Ser Phe Ser Gln Ser Glu
500          505          510

tac ctc ata tac aag gag agc cag tgt cgc ctg cgc tac ctg ctg gag 1701
Tyr Leu Ile Tyr Lys Glu Ser Gln Cys Arg Leu Arg Tyr Leu Leu Glu
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Asp Cys Thr Leu Asn Gln Thr 65	Asn Ile Gly Asn Asn Asn Asn Lys 70	Phe 80
Tyr Ile Ile Gln Leu Leu Glu Glu Gly 85	Ser Arg Phe Phe Cys Trp 90	Asn 95
Arg Trp Gly Arg Val Gly Glu Val 100	Gly Gln Ser Lys Met Asn His 105	Phe 110
Thr Cys Leu Glu Asp Ala Lys Lys 115	Asp Phe Lys Lys Lys Phe Trp 120	Glu 125
Lys Thr Lys Asn Lys Trp Glu Glu Arg 130	Asp Arg Phe Val Ala Gln Pro 135	
Asn Lys Tyr Thr Leu Ile Glu Val Gln 145	Gly Glu Ala Glu Ser Gln Glu 150	
Ala Val Val Lys Ala Leu Ser Pro Gln 165	Val Asp Ser Gly Pro Val Arg 170	
Thr Val Val Lys Pro Cys Ser Leu 180	Asp Pro Ala Thr Gln Asn Leu Ile 185	
Thr Asn Ile Phe Ser Lys Glu Met 195	Phe Lys Asn Ala Met Thr Leu Met 200	
Asn Leu Asp Val Lys Lys Met Pro Leu 210	Gly Lys Leu Thr Lys Gln Gln 215	
Ile Ala Arg Gly Phe Glu Ala Leu Glu 225	Ala Leu Glu Glu Ala Met Lys 230	
Asn Pro Thr Gly Asp Gly Gln Ser Leu 245	Glu Glu Leu Ser Ser Cys Phe 250	
Tyr Thr Val Ile Pro His Asn Phe 260	Gly Arg Ser Arg Pro Pro Pro Ile 265	
Asn Ser Pro Asp Val Leu Gln Ala 275	Lys Lys Asp Met Leu Leu Val Leu 280	
Ala Asp Ile Glu Leu Ala Gln Thr 290	Leu Gln Ala Ala Pro Gly Glu Glu 295	
Glu Glu Lys Val Glu Glu Val Pro His 305	Pro Leu Asp Arg Asp Tyr Gln 310	
Leu Leu Arg Cys Gln Leu Gln Leu Leu 325	Asp Ser Gly Glu Ser Glu Tyr 330	
Lys Ala Ile Gln Thr Tyr Leu Lys Gln 340	Thr Gly Asn Ser Tyr Arg Cys 345	
Pro Asn Leu Arg His Val Trp Lys Val 355	Asn Arg Glu Gly Glu Gly Asp 360	

2330206.seq

Arg Phe Gln Ala His Ser Lys Leu Gly Asn Arg Arg Leu Leu Trp His
 370 375 380
 Gly Thr Asn Val Ala Val Val Ala Ala Ile Leu Thr Ser Gly Leu Arg
 385 390 395 400
 Ile Met Pro His Ser Gly Gly Arg Val Gly Lys Gly Ile Tyr Phe Ala
 405 410 415
 Ser Glu Asn Ser Lys Ser Ala Gly Tyr Val Thr Thr Met His Cys Gly
 420 425 430
 Gly His Gln Val Gly Tyr Met Phe Leu Gly Glu Val Ala Leu Gly Lys
 435 440 445
 Glu His His Ile Thr Ile Asp Asp Pro Ser Leu Lys Ser Pro Pro Pro
 450 455 460
 Gly Phe Asp Ser Val Ile Ala Arg Gly Gln Thr Glu Pro Asp Pro Ala
 465 470 475 480
 Gln Asp Ile Glu Leu Glu Leu Asp Gly Gln Pro Val Val Val Pro Gln
 485 490 495
 Gly Pro Pro Val Gln Cys Pro Ser Phe Lys Ser Ser Ser Phe Ser Gln
 500 505 510
 Ser Glu Tyr Leu Ile Tyr Lys Glu Ser Gln Cys Arg Leu Arg Tyr Leu
 515 520 525
 Leu Glu Ile His Leu
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 Gln Arg Gln Gly Thr Glu Glu Glu Asp Ser Phe Arg Ser Thr Ala Glu
 20 25 30
 gct ctc aga gca gca cct gct gat aat cgg gtc atc cgt gtg gac ccc 144
 Ala Leu Arg Ala Ala Pro Ala Asp Asn Arg Val Ile Arg Val Asp Pro
 35 40 45
 tca tgt cca ttc agc cgg aac ccc ggg ata cag gtc cac gag gac tat 192
 Ser Cys Pro Phe Ser Arg Asn Pro Gly Ile Gln Val His Glu Asp Tyr
 50 55 60
 gac tgt acc ctg aac cag acc aac atc ggc aac aac aac aac aag ttc 240
 Asp Cys Thr Leu Asn Gln Thr Asn Ile Gly Asn Asn Asn Asn Lys Phe

2330206.seq

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cgc tgg ggc cgc gtg gga gag gtg ggc cag agc aag atg aac cac ttc				336
Arg Trp Gly Arg Val Gly Glu Val Gly Gln Ser Lys Met Asn His Phe	100	105	110	
acc tgc ctg gaa gat gca aag aag gac ttt aag aag aaa ttt tgg gag				384
Thr Cys Leu Glu Asp Ala Lys Lys Asp Phe Lys Lys Lys Phe Trp Glu	115	120	125	
aag act aaa aac aaa tgg gag gag cgg gac cgt ttt gtg gcc cag ccc				432
Lys Thr Lys Asn Lys Trp Glu Glu Arg Asp Arg Phe Val Ala Gln Pro	130	135	140	
aac aag tac aca ctt ata gaa gtc cag gga gaa gca gag agc caa gag				480
Asn Lys Tyr Thr Leu Ile Glu Val Gln Gly Glu Ala Glu Ser Gln Glu	145	150	155	160
gct gta gtg aag gtg gac agc ggc cct gtg agg acc gtg gtc aag ccc				528
Ala Val Val Lys Val Asp Ser Gly Pro Val Arg Thr Val Val Lys Pro	165	170	175	
tgc tcc cta gac cct gcc acc cag aac ctt atc acc aac atc ttc agc				576
Cys Ser Leu Asp Pro Ala Thr Gln Asn Leu Ile Thr Asn Ile Phe Ser	180	185	190	
aaa gag atg ttc aag aac gca atg acc ctc atg aac ctg gat gtg aag				624
Lys Glu Met Phe Lys Asn Ala Met Thr Leu Met Asn Leu Asp Val Lys	195	200	205	
aag atg ccc ttg gga aag ctg acc aag cag cag att gcc cgt ggc ttc				672
Lys Met Pro Leu Gly Lys Leu Thr Lys Gln Gln Ile Ala Arg Gly Phe	210	215	220	
gag gcc ttg gaa gct cta gag gag gcc atg aaa aac ccc aca ggg gat				720
Glu Ala Leu Glu Ala Leu Glu Glu Ala Met Lys Asn Pro Thr Gly Asp	225	230	235	240
ggc cag agc ctg gaa gag ctc tcc tcc tgc ttc tac act gtc atc cca				768
Gly Gln Ser Leu Glu Glu Leu Ser Ser Cys Phe Tyr Thr Val Ile Pro	245	250	255	
cac aac ttc ggc cgc agc cga ccc ccg ccc atc aac tcc cct gat gtg				816
His Asn Phe Gly Arg Ser Arg Pro Pro Pro Ile Asn Ser Pro Asp Val	260	265	270	
ctt cag gcc aag aag gac atg ctg ctg gtg cta gcg gac atc gag ttg				864
Leu Gln Ala Lys Lys Asp Met Leu Leu Val Leu Ala Asp Ile Glu Leu	275	280	285	
gcg cag acc ttg cag gca gcc cct ggg gag gag gag gag aaa gtg gaa				912
Ala Gln Thr Leu Gln Ala Ala Pro Gly Glu Glu Glu Glu Lys Val Glu	290	295	300	
gag gtg cca cac cca ctg gat cga gac tac cag ctc ctc agg tgc cag				960
Glu Val Pro His Pro Leu Asp Arg Asp Tyr Gln Leu Leu Arg Cys Gln	305	310	315	320
ctt caa ctg ctg gac tcc ggg gag tcc gag tac aag gca ata cag acc				1008
Leu Gln Leu Leu Asp Ser Gly Glu Ser Glu Tyr Lys Ala Ile Gln Thr				

2330206.seq

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340	345	350	
gtt tgg aaa gtg aac cga gaa ggg	gag gga gac agg ttc cag gcc cac		1104
Val Trp Lys Val Asn Arg Glu Gly	Glu Gly Asp Arg Phe Gln Ala His		
355	360	365	
tcc aaa ctg ggc aat cgg agg ctg	ctg tgg cac ggc acc aat gtg gcc		1152
Ser Lys Leu Gly Asn Arg Arg Leu	Leu Trp His Gly Thr Asn Val Ala		
370	375	380	
gtg gtg gct gcc atc ctc acc agt	ggg ctc cga atc atg cca cac tcg		1200
Val Val Ala Ala Ile Leu Thr Ser	Gly Leu Arg Ile Met Pro His Ser		
385	390	395	400
ggg ggt cgt gtt ggc aag ggt att	tat ttt gcc tct gag aac agc aag		1248
Gly Gly Arg Val Gly Lys Gly Ile	Tyr Phe Ala Ser Glu Asn Ser Lys		
405	410	415	
tca gct ggc tat gtt acc acc atg	cac tgt ggg ggc cac cag gtg ggc		1296
Ser Ala Gly Tyr Val Thr Thr Met	His Cys Gly Gly His Gln Val Gly		
420	425	430	
tac atg ttc ctg ggc gag gtg gcc	ctc ggc aaa gag cac cac atc acc		1344
Tyr Met Phe Leu Gly Glu Val Ala	Leu Gly Lys Glu His His Ile Thr		
435	440	445	
atc gat gac ccc agc ttg aag agt	cca ccc cct ggc ttt gac agc gtc		1392
Ile Asp Asp Pro Ser Leu Lys Ser	Pro Pro Pro Gly Phe Asp Ser Val		
450	455	460	
atc gcc cga ggc caa acc gag ccg	gat ccc gcc cag gac att gaa ctt		1440
Ile Ala Arg Gly Gln Thr Glu Pro	Asp Pro Ala Gln Asp Ile Glu Leu		
465	470	475	480
gaa ctg gat ggg cag ccg gtg gtg	gtg ccc caa ggc ccg cct gtg cag		1488
Glu Leu Asp Gly Gln Pro Val Val	Val Pro Gln Gly Pro Pro Val Gln		
485	490	495	
tgc ccg tca ttc aaa agc tcc agc	ttc agc cag agt gaa tac ctc ata		1536
Cys Pro Ser Phe Lys Ser Ser Ser	Phe Ser Gln Ser Glu Tyr Leu Ile		
500	505	510	
tac aag gag agc cag tgt cgc ctg	cgc tac ctg ctg gag att cac ctc		1584
Tyr Lys Glu Ser Gln Cys Arg Leu	Arg Tyr Leu Leu Glu Ile His Leu		
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taa			1587

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 Asp Cys Thr Leu Asn Gln Thr Asn Ile Gly Asn Asn Asn Asn Lys Phe
 65 70 75 80
 Tyr Ile Ile Gln Leu Leu Glu Glu Gly Ser Arg Phe Phe Cys Trp Asn
 85 90 95
 Arg Trp Gly Arg Val Gly Glu Val Gly Gln Ser Lys Met Asn His Phe
 100 105 110
 Thr Cys Leu Glu Asp Ala Lys Lys Asp Phe Lys Lys Lys Phe Trp Glu
 115 120 125
 Lys Thr Lys Asn Lys Trp Glu Glu Arg Asp Arg Phe Val Ala Gln Pro
 130 135 140
 Asn Lys Tyr Thr Leu Ile Glu Val Gln Gly Glu Ala Glu Ser Gln Glu
 145 150 155 160
 Ala Val Val Lys Val Asp Ser Gly Pro Val Arg Thr Val Val Lys Pro
 165 170 175
 Cys Ser Leu Asp Pro Ala Thr Gln Asn Leu Ile Thr Asn Ile Phe Ser
 180 185 190
 Lys Glu Met Phe Lys Asn Ala Met Thr Leu Met Asn Leu Asp Val Lys
 195 200 205
 Lys Met Pro Leu Gly Lys Leu Thr Lys Gln Gln Ile Ala Arg Gly Phe
 210 215 220
 Glu Ala Leu Glu Ala Leu Glu Glu Ala Met Lys Asn Pro Thr Gly Asp
 225 230 235 240
 Gly Gln Ser Leu Glu Glu Leu Ser Ser Cys Phe Tyr Thr Val Ile Pro
 245 250 255
 His Asn Phe Gly Arg Ser Arg Pro Pro Pro Ile Asn Ser Pro Asp Val
 260 265 270
 Leu Gln Ala Lys Lys Asp Met Leu Leu Val Leu Ala Asp Ile Glu Leu
 275 280 285
 Ala Gln Thr Leu Gln Ala Ala Pro Gly Glu Glu Glu Glu Lys Val Glu
 290 295 300
 Glu Val Pro His Pro Leu Asp Arg Asp Tyr Gln Leu Leu Arg Cys Gln
 305 310 315 320
 Leu Gln Leu Leu Asp Ser Gly Glu Ser Glu Tyr Lys Ala Ile Gln Thr
 325 330 335
 Tyr Leu Lys Gln Thr Gly Asn Ser Tyr Arg Cys Pro Asn Leu Arg His
 340 345 350
 Val Trp Lys Val Asn Arg Glu Gly Glu Gly Asp Arg Phe Gln Ala His
 355 360 365

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Ser Lys Leu Gly Asn Arg Arg Leu Leu Trp His Gly Thr Asn Val Ala
 370 375 380
 Val Val Ala Ala Ile Leu Thr Ser Gly Leu Arg Ile Met Pro His Ser
 385 390 395 400
 Gly Gly Arg Val Gly Lys Gly Ile Tyr Phe Ala Ser Glu Asn Ser Lys
 405 410 415
 Ser Ala Gly Tyr Val Thr Thr Met His Cys Gly Gly His Gln Val Gly
 420 425 430
 Tyr Met Phe Leu Gly Glu Val Ala Leu Gly Lys Glu His His Ile Thr
 435 440 445
 Ile Asp Asp Pro Ser Leu Lys Ser Pro Pro Pro Gly Phe Asp Ser Val
 450 455 460
 Ile Ala Arg Gly Gln Thr Glu Pro Asp Pro Ala Gln Asp Ile Glu Leu
 465 470 475 480
 Glu Leu Asp Gly Gln Pro Val Val Val Pro Gln Gly Pro Pro Val Gln
 485 490 495
 Cys Pro Ser Phe Lys Ser Ser Ser Phe Ser Gln Ser Glu Tyr Leu Ile
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<210> 12
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 <213> Artificial sequence

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2330206.seq

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<220>

<221> SITE

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<223> Xaa is Ile or Val

<220>

<221> SITE

<222> (9)

<223> Xaa is 1 to 5 other amino acids

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<221> SITE

<222> (10)

<223> Xaa is Ser or Thr

<220>

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Gly	Ile	Tyr	Phe	Ala
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<222> (6)

<223> Xaa is Ser or Thr

<220>

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<222> (16)

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<220>

<221> SITE

<222> (21)

<223> Xaa is Ile or Val

<220>

<221> SITE

<222> (24)

<223> Xaa is 1 to 5 other amino acids

<220>

<221> SITE

<222> (25)

<223> Xaa is Ser or Thr

<400> 13

Leu	Leu	Trp	His	Gly	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Ile	Leu	Xaa
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

2330206.seq

1	5	10	15
Xaa Gly Leu Arg Xaa Xaa Pro Xaa Xaa Gly Xaa Xaa Xaa Gly Lys Gly	20	25	30
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<220>
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<400> 14

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Leu Xaa	1	5	10	15
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Xaa Xaa Xaa Xaa Xaa Leu
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<210> 15
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<220>
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<220>
 <223> Description of artificial sequence: peptide

<400> 15

Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Tyr Xaa Xaa	1	5	10	15
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Gln Leu Leu Xaa Xaa Xaa Trp Gly Arg Val Gly
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Trp Xaa Xaa Xaa Xaa Xaa Phe Xaa Xaa Xaa Pro Xaa Lys
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Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro Leu Gly Lys Leu
 20 25 30

Xaa Xaa Xaa Gln Ile Xaa Xaa Xaa Xaa Xaa Xaa Leu
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<210> 18

<211> 15

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Phe Tyr Thr Xaa Ile Pro His Xaa Phe Gly Xaa Xaa Xaa Pro Pro
 1 5 10 15

<210> 19

<211> 17

<212> PRT

<213> Artificial sequence

<220>

<223> Description of artificial sequence: peptide

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Lys Xaa Xaa Xaa Leu Xaa Xaa Leu Xaa Asp Ile Glu Xaa Ala Xaa Xaa
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Leu

2330206.seq

<210> 20
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Gly Xaa Xaa Val
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2330206.seq

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Leu Asn Glu Ser
 20

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Lys Thr Glu Leu Gln Ser Pro Glu His Pro Leu Asp Gln His Tyr Arg
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Asn Leu His Cys
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<210> 25

<211> 21

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Cys Lys Gly Arg Gln Ala Gly Arg Glu Glu Asp Pro Phe Arg Ser Thr
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Ala Glu Ala Leu Lys
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<210> 26

<211> 20

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<223> Description of artificial sequence: peptide

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Cys Lys Gln Gln Ile Ala Arg Gly Phe Glu Ala Leu Glu Ala Leu Glu
 1 5 10 15

Glu Ala Leu Lys
 20

<210> 27

2330206.seq

<211> 19

<212> PRT

<213> Artificial sequence

<220>

<223> Description of artificial sequence: peptide

<400> 27

Lys	Gln	Gln	Ile	Ala	Arg	Gly	Phe	Glu	Ala	Leu	Glu	Ala	Leu	Glu	Glu
1				5				10				15			

Ala Leu Lys

<210> 28

<211> 19

<212> PRT

<213> Artificial sequence

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<223> Description of artificial sequence: peptide

<400> 28

Lys	Gln	Gln	Ile	Ala	Arg	Gly	Phe	Glu	Ala	Leu	Glu	Ala	Leu	Glu	Glu
1				5				10				15			

Ala Met Lys

WHAT IS CLAIMED IS:

1. A poly-(ADP-ribose)-polymerase (PARP) homologue or a functional equivalents thereof which is at least 85% homologous thereto, characterized by an amino acid sequence comprising

- a) a functional NAD⁺ binding domain and
- b) no zinc finger sequence motif of the general formula

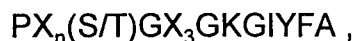


wherein

10 m is an integer of 28 or 30 and

X is, independently at each occurrence, any amino acid residue;

wherein the functional NAD⁺ binding domain comprises the motif



wherein

n is an integer from 1 to 5 and

X is, independently at each occurrence, any amino acid residue.

2. The PARP homologue according to claim 1, characterized in that the functional NAD⁺ binding domain comprises one of the following general sequence motifs:

20 (S/T)XGLR(I/V)XPX_n(S/T)GX₃GKGIYFA or

LLWHG(S/T)X₇IL(S/T)XGLR(I/V)XPX_n(S/T)GX₃GKGIYFAX₃SKSAXY,

wherein n and X are as defined in claim 1.

3. The PARP homologue according to claim 1 or 2, comprising at least one of the following partial sequence motifs:

LX₉NX₂YX₂QLLX(D/E)X_{10/11}WGRVG,

AX₃FXKX₄KTXNXWX₅FX₃PXK,

QXL(I/L)X₂IX₉MX₁₀PLGKLX₃QIX₆L,
 FYTXIPHXFGX₃PP; and
 KX₃LX₂LXDIEXAX₂L,

wherein X is, independently at each occurrence, any amino acid residue.

4. The PARP homologue according to any one of claims 1 to 3, selected among the group consisting of human PARP homologues comprising the amino acid sequences of SEQ ID NO:2, SEQ ID NO:4 or 6; and murine PARP homologues, comprising amino acid sequences of SEQ ID NO:8 or SEQ ID NO:10.
5. A binding partner for a PARP homologue according to any one of claims 1 to 4, selected among the group consisting of antibodies and fragments thereof, wherein the antibodies and fragments thereof bind with specificity to the PARP homologue.
6. A nucleic acid, comprising
 - a) a nucleotide sequence encoding at least one PARP homologue according to any one of claims 1 to 4, or the complementary nucleotide sequence thereof;
 - b) a nucleotide sequence which hybridizes with a sequence as specified in a) under stringent conditions wherein the hybridization mixture is washed with 0.1X SSC buffer (20X SSC buffer is 3M NaCl, 0.3M sodium citrate, pH 7.0) and 0.1% SDS at a temperature of about 60°C; or
 - c) a nucleotide sequence derived from the nucleotide sequences defined in a) and b) through the degeneracy of the genetic code.
7. The nucleic acid according to claim 6, comprising
 - a) nucleotides +3 to +1715 according to SEQ ID NO:1;
 - b) nucleotides +242 to +1843 according to SEQ ID NO:3;
 - c) nucleotides +221 to +1843 according to SEQ ID NO:5;

- d) nucleotides +112 to +1710 according to SEQ ID NO:7;
- e) nucleotides +1 to +1584 according to SEQ ID NO:9.

8. An expression cassette, comprising at least one nucleotide sequence according to claim 6 or 7 under the genetic control of at least one regulatory nucleotide sequence.

9. A recombinant vector, comprising at least one expression cassette according to claim 8.

10. A recombinant microorganism, comprising at least one recombinant vector according to claim 9.

10 11. An *in vitro* method for identifying PARP inhibitors, characterized in that it comprises

- a) incubating a carrier-coupled or non-carrier-coupled poly-ADP-ribosylable target with a reaction mixture, comprising
 - a1) a PARP homologue according to any one of claims 1 to 4,
 - a2) damaged or simulated damaged DNA as PARP activator; and
 - a3) a PARP inhibitor or an analyte suspected to comprise at least one PARP inhibitor;
- b) performing the poly-ADP ribosylation reaction;
- c) qualitatively or quantitatively determining the target's poly-ADP
20 ribosylation; and
- d) detecting PARP inhibitors.

12. The method according to claim 11, characterized in that the PARP homologue is preincubated with the PARP activator and the PARP inhibitor or an analyte suspected to comprise at least one PARP inhibitor prior to performing the poly-ADP ribosylation reaction.

13. The method according to claim 11 or 12, characterized in that the poly-ADP-ribosylable target is a histone protein.
14. The method according to any one of claims 11 to 13, characterized in that the poly-ADP ribosylation reaction is initiated by addition of NAD^+ .
15. The method according to any one of claims 11 to 14, characterized in that the poly-ADP ribosylation of the carrier-coupled target is determined using an anti-poly-(ADP-ribose) antibody.
16. The method according to any one of claims 11 to 14, characterized in that the non-carrier-coupled target is labeled with an acceptor fluorophore.
- 10 17. The method according to claim 16, characterized in that the poly-ADP ribosylation of the non-carrier-coupled target is determined using anti-poly-(ADP-ribose) antibody labeled with a donor fluorophore capable of transferring energy to the acceptor fluorophore.
18. The method according to claim 16 or 17, characterized in that the target is biotinylated histone and the acceptor fluorophore is coupled to it via avidine or streptavidine.
19. The method according to claim 17 or 18, characterized in that the anti-poly-(ADP-ribose) antibody carries a europium cryptate as donor fluorophore.
20. An *in vitro* screening method for binding partners for a PARP molecule, characterized in that it comprises
 - a1) immobilizing at least one PARP homologue according to any one of claims 1 to 4 to a carrier;
 - b1) contacting the immobilized PARP homologue with an analyte suspected to comprise at least one PARP inhibitor; and

- c1) determining the components of the analyte bound to the immobilized PARP homologue; or
- a2) immobilizing an analyte suspected to comprise at least one PARP inhibitor to a carrier;
- b2) contacting the immobilized analyte with at least one PARP homologue according to any one of claims 1 to 4 which a binding partner is sought for; and
- c2) assaying the immobilized analyte for binding of the PARP homologue.

21. The method according to claim 20, characterized in that steps c1) and/or c2)
10 are performed after a period of incubation.

22. A method for qualitatively or quantitatively determining nucleic acids encoding PARP homologues according to any one of claims 1 to 4, characterized in that it comprises

- a) incubating a biological sample with a defined amount of an exogenous nucleic acid according to claim 6 or 7, hybridizing under stringent conditions wherein the hybridization mixture is washed with 0.1X SSC buffer (20X SSC buffer is 3M NaCl, 0.3M sodium citrate, pH 7.0) and 0.1% SDS at a temperature of about 60°C, and detecting the hybridizing nucleic acids; or
- 20 b) incubating a biological sample with a pair of oligonucleotide primers with specificity for a nucleic acid encoding the PARP homologue according to claim 6 or 7, amplifying the nucleic acid, and detecting the amplification product.

23. The method according to claim 22, characterized in that a) further includes comparing the result to a reference standard.

24. The method according to claim 22 or 23, characterized in that b) further includes comparing the result to a reference standard.
25. A method for qualitatively or quantitatively determining a PARP homologue according to any one of claims 1 to 4, characterized in that it comprises
- a) incubating a biological sample with a binding partner for the PARP homologue according to claim 5, and
 - b) detecting the binding partner/PARP complex.
26. The method according to claim 25, further comprising a step of comparing the result to a reference standard.
- 10 27. The method according to claim 25 or 26, characterized in that the binding partner is an antibody or a binding fragment thereof.
28. The method according to claim 27, characterized in that the binding partner carries a detectable label.
29. The method according to any one of claims 22 to 28 for diagnosis of disorders mediated by energy deficiency, said disorders comprising ischemia/reperfusion damage, vascular stroke, age-related macular degeneration, immunodeficiency disorders, arthritis, atherosclerosis, cachexia, cancer, degenerative disorders of the skeletal muscles, diabetes, cranial trauma, inflammatory disorders of the gastrointestinal tract, muscular dystrophy,
- 20 osteoporosis, chronic pain, acute pain, kidney failure, septic shock and aging.
30. A method for determining the efficiency of PARP inhibitors, characterized in that it comprises
- a) incubating a PARP homologue according to any one of claims 1 to 4 with an analyte comprising a PARP inhibitor; and
 - b) determining the activity of the PARP homologue.

31. The method according to claim 30, characterized in that the inhibitor is removed before b).
32. The method according to claim 30 or 31, characterized in that b) is performed after adding substrates or cosubstrates.
33. A composition for gene therapy, characterized in that it comprises, within a carrier acceptable for gene therapy, a nucleic acid construct encoding an antisense nucleic acid for coding a nucleic acid according to claim 6 or 7.
34. A pharmaceutical composition, comprising, within a pharmaceutically acceptable carrier, at least one PARP protein according to any one of claims 1 to 4,
10 at least one PARP binding partner according to claim 5 or at least one coding nucleotide sequence according to claim 6 or 7.
35. Use of a PARP binding partner according to claim 5 for the manufacture of a pharmaceutical composition for the diagnosis or therapy of disorders mediated by energy deficiency, said disorders comprising ischemia/reperfusion damage, vascular stroke, age-related macular degeneration, immunodeficiency disorders, arthritis, atherosclerosis, cachexia, cancer, degenerative disorders of the skeletal muscles, diabetes, cranial trauma, inflammatory disorders of the gastrointestinal tract, muscular dystrophy, osteoporosis, chronic pain, acute pain, kidney failure, septic shock and aging.

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Majority												Majority	
1	MA	10	20	30	40	50	60						humanPARP1
1	MA	10	20	30	40	50	60						humanPARP2
1	MA	10	20	30	40	50	60						humanPARP3
1	MA	10	20	30	40	50	60						murinePARP
1	MA	10	20	30	40	50	60						Majority
61	GH	70	80	90	100	110	120						humanPARP1
5	GH	70	80	90	100	110	120						humanPARP2
3	GH	70	80	90	100	110	120						humanPARP3
2	GH	70	80	90	100	110	120						murinePARP
2	GH	70	80	90	100	110	120						Majority
121	HR	130	140	150	160	170	180						humanPARP1
20	HR	130	140	150	160	170	180						humanPARP2
3	HR	130	140	150	160	170	180						humanPARP3
2	HR	130	140	150	160	170	180						murinePARP
2	HR	130	140	150	160	170	180						Majority
181	LR	190	200	210	220	230	240						humanPARP1
43	LR	190	200	210	220	230	240						humanPARP2
3	LR	190	200	210	220	230	240						humanPARP3
2	LR	190	200	210	220	230	240						murinePARP
2	LR	190	200	210	220	230	240						Majority
241	QR	250	260	270	280	290	300						humanPARP1
68	QR	250	260	270	280	290	300						humanPARP2
3	QR	250	260	270	280	290	300						humanPARP3
2	QR	250	260	270	280	290	300						murinePARP
2	QR	250	260	270	280	290	300						Majority

Fig. 1(1)

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	Y C G	A P K R K X W V	Majority
301	Q L V P K S D A Y Y C T G D V T A W T K C M V K T Q T P N R K E W V T P K E F R E I S Y L K K L K V K K Q D R I F P P E	360	humanPARP1
88	K V G - - K A H V Y C E G N - - - - -	350	humanPARP2
9	- - - - -	340	humanPARP3
2	- - - - -	330	murinePARP
	T E G S	Majority	
361	T S A S V A A T P P S T A S A P A A V H S S A S A D K P L S N H K I L T L G K L S R N K D E V K A M I E K L G G K L T	420	humanPARP1
100	- - - - -	410	humanPARP2
18	T E G P - - - - -	400	humanPARP3
11	T E G S - - - - -	390	murinePARP
	- - - - -	Majority	
421	G T A N K A S L C I S T K K E V E K H N K K M E E V K B A N I R V V S E D F L Q D V S A S T K S L Q E L F L A H I L S P	480	humanPARP1
100	- - - - -	470	humanPARP2
22	- - - - -	460	humanPARP3
15	- - - - -	450	murinePARP
	- - - - -	Majority	
481	K A X P A E X R X I R V D P X C P L S X N P O X Q V X E D - - - - -	540	humanPARP1
100	- - - - -	530	humanPARP2
44	- - - - -	520	humanPARP3
35	- - - - -	510	murinePARP
	- - - - -	Majority	
541	H S A H V L E K G G K V F S A T L G L V D I V K G T N S Y Y K L Q L L E D D K E N R Y W I F R S W G R V G T V I Q S N K	600	humanPARP1
100	- - - - -	590	humanPARP2
73	- - - - -	580	humanPARP3
64	- - - - -	570	murinePARP

Fig. 1(2)

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601	L N H F T X - L E D A K K E D F X K K F X E K T K N N W E E R D X F V K X P G K Y T L L E V D Y - X E X E D E E A V V K - Majority	610	620	630	640	650	660
149	L E Q H F S K - E D A I E H F H K K L Y E E K T G N A W H S K N - F T K Y P K K P Y P L E I D Y G - - Q D E E A V K K - humanPARP1						
119	L V A C S G H L N K A K E I P O K K F L D K T K N N W E D R E K F E K V P Q K Y D H L Q H D Y A T N T Q D E E T K K E humanPARP2						
109	I N H F T R - L E D A K K D P E K K F R E K T K N N W A E R D R F V S H P Q K Y T L I E V Q - - A E D E A Q E A V V K - humanPARP3						
	H N H F T C - L E D A K K D P K K K F W E K T K N N W E E R D R F V A Q P N K Y T L I E V Q - - G E A E S Q E A V V K - murinePARP						
	- S L X V D X G P V S T V X K R V Q P C S L D P A T Q X L I T N I F S V E M F K N A M X L M X L D V K K M P L G K L S K Majority	670	680	690	700	710	720
655	- L T V N P G T K S K L P K P V Q - - - - - D L I K H I P D V E S H K K A H V E Y E I D L Q K M P L G K L S K humanPARP1						
209	E S L K S P L K P E S Q L D L R V O - - - - - E L I K L I C H V Q A H E E M H M E H K Y N T K K A P L G K L T V humanPARP2						
175	- - - V D R G P V R T V T K R V Q P C S L D P A T Q K L I T N I F S K E M F K N T M A L H D L D V K K M P L G K L S K humanPARP3						
166	L S P O V D S G P V R T V V K - - - P C S L D P A T Q N L I T N I F S K E M F K N A H T L M N L D V K K M P L G K L T K murinePARP						
	Q Q I A A G F E A L E A L E E A X K X G T X G G Q S L E E L S S X P Y T V I P H D F G X S X P P L I N S P D X L Q A K K Majority	730	740	750	760	770	780
704	R O I A A Y S I L S E V Q Q A V S Q S S D S Q I L D - L S N R F Y T L I P H D P G M K K P P L L N N A D S V Q A K V humanPARP1						
260	A Q I K A G Y Q S L K K I E D C I R A G Q H G R A L N E - A C H E F Y T R I P H D F G L R T P P L I R T Q K E L S E K I humanPARP2						
231	Q O I A R G F E A L E A L E E A L K G P T D G Q Q S L E E L S S H F Y T V I P H N F O H S Q P P P I N S P E L L Q A K K humanPARP3						
223	Q O I A R G F E A L E A L E E A L K G P T D G Q Q S L E E L S S C F Y T V I P H N F G R S R P P P I N S P D V L Q A K K murinePARP						
	D H L L V L A D I E L A Q X L Q A X X E X S X K V E E V P H P L D R D Y Q L L K C Q L Q L L D S Q S X E Y K V I Q T Y Majority	790	800	810	820	830	840
763	E M L D N L L D I E V A Y S L L R G G S D D S S K - - - - - D P I D V N V E K L K T D I K V V D R D S E A E I I R K Y humanPARP1						
319	Q L L E A L G D I E I A I K L V K T B L Q - S P E - - - - - H P L D D Q H Y R N L H C A L R P L D H E L S Y E F K V I S O Y humanPARP2						
291	D M L L V L A D I E L A Q A L Q A V S - E Q E K T V E E V P H P L D R D Y Q L L K C Q L Q L L D S O A P E Y K V I Q T Y humanPARP3						
283	D M L L V L A D I E L A Q A L Q A V S - E Q E K T V E E V P H P L D R D Y Q L L K C Q L Q L L D S O A P E Y K V I Q T Y murinePARP						
	L K Q T G A X T H C F Y - - T L X D I P K V R R E G E X D R F Q A H S K L O H R R L L W H O S N M A V V A Q I L S S G L Majority	850	860	870	880	890	900
818	V K N T H A T T H N A Y D L E V I D I F K I E R E G E C Q R Y K P P K Q L H N R R L L W H G S R T T N F A Q I L S Q Q L humanPARP1						
373	L O S T H A P T H S D Y T T M T L L D L P E V E K D G E K E A F R - - E D L H N R R L L W H G S R M S N W V Q I L S H G L humanPARP2						
350	L E Q T G S N H R C P - - - T L Q H I W K V N Q E E E D R P O A H S K L Q H R R L L W H G T N M A V V A I L T S Q L humanPARP3						
343	L K O T G S N S Y R C P - - - N L R H V W K V N R E G E G D R P O A H S K L Q H R R L L W H G T N V A V V A I L T S G L murinePARP						

Fig. 1(3)

Fig. 1(4)

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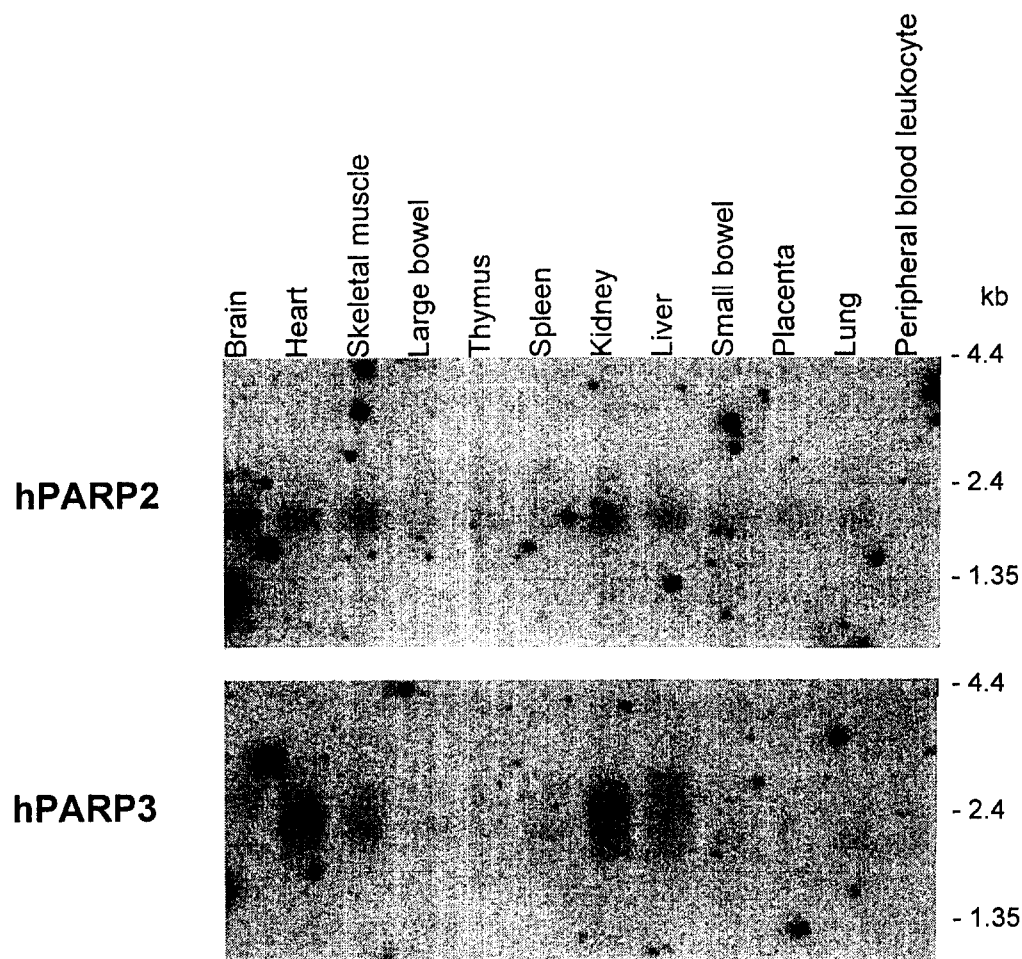
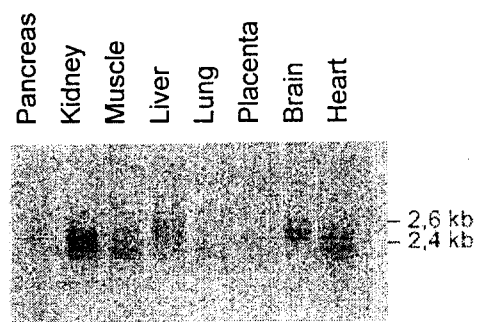
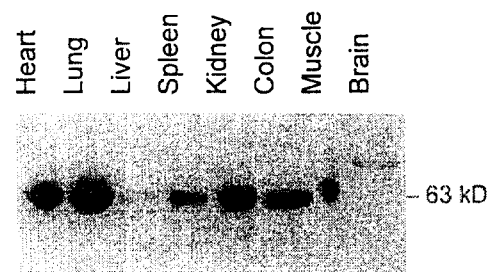


Fig. 2



Northern Blot Analysis
Human PARP3 Tissue Distribution



Western Blot Analysis
PARP3 Tissue Distribution

Fig. 3

Fig. 4

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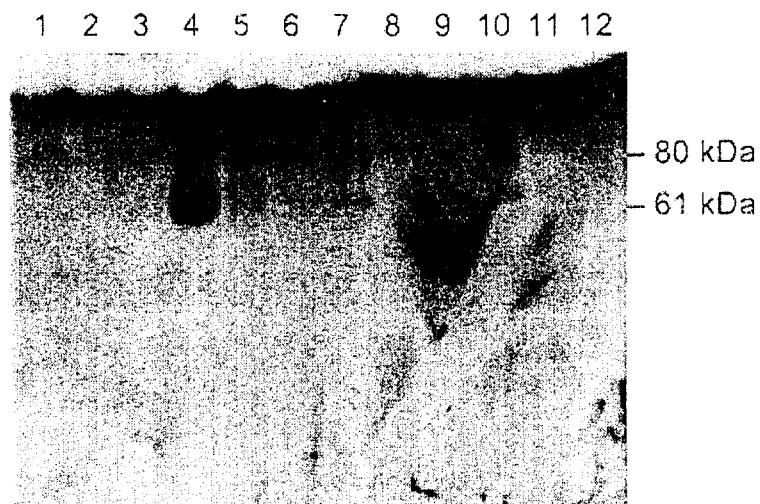
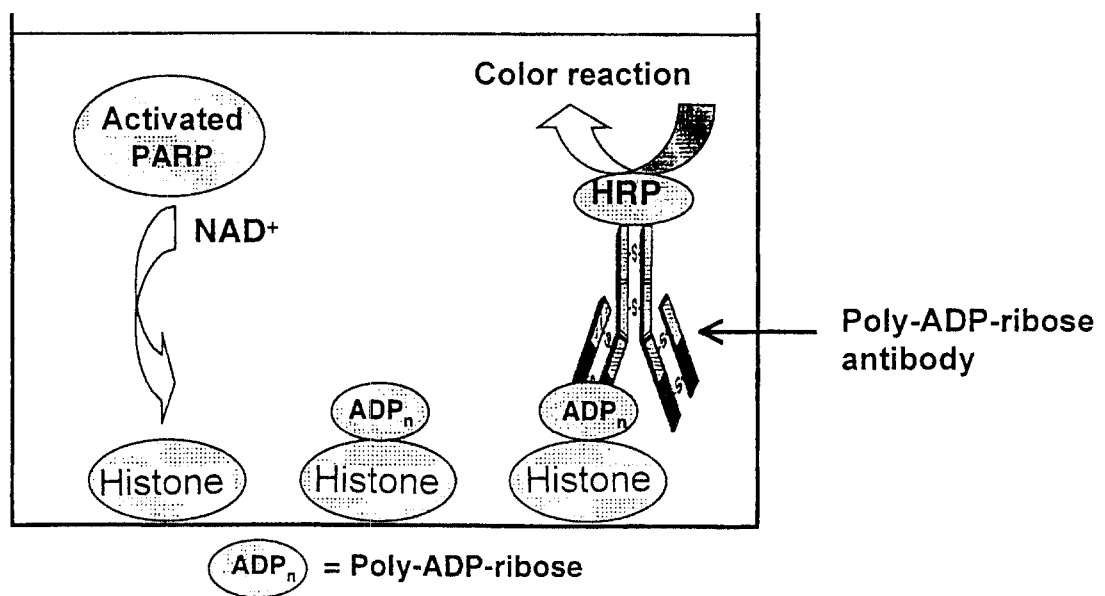


Fig. 5



HRP = Horseradish-Peroxidase

Fig. 6

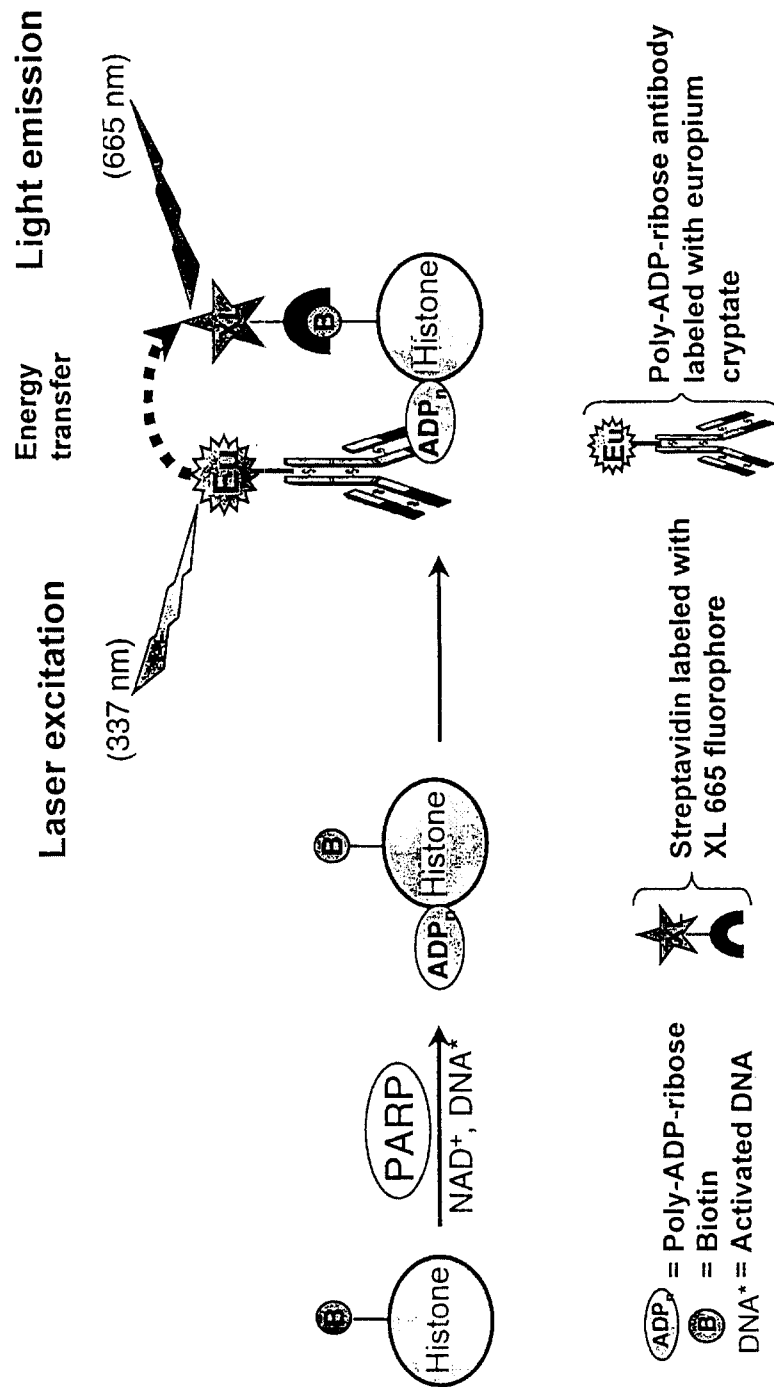


Fig. 7